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Tuesday. August 22

1 Drugs and Membranes. Introductory Remarks. C. A. M. Hogben (U.S.A.).

2 The Molecular Architecture of Cell Membrane and Cytoplasmic Membranes. F. S. Sjöstrand (U.S.A.).

Indirect methods revealing certain properties of the cell membrane combined with direct morphological analysis of the structure of various types of membranes by means of high resolution electron microscopy have allowed proposing models for the molecular architecture of these membranes. Electron microscopy, furthermore, can demonstrate structural changes in the membranes that can be correlated to variations in functional condition. The hypothesis will be discussed according to which the membranes represent enzymatically active structures involved in, for instance, oxidative phosphorylation in mitochondria and protein synthesis in certain other cytoplasmic membranes.

Membranes of various types represent the most common and spectacular structural components of the cytoplasm of cells and might be of great importance for co-ordination and regulation of the enzymatic activities of cells. The hypothesis regarding their functional importance can be tested. The role of the various membranes in connection with the action of drugs could be further explored by combined biochemical and morphological analysis. Possible approaches to this problem will be discussed.

3 Passage of Materials across Biological Membranes. H. H. Ussing (Denmark).

A survey of current research on cell permeability indicates the lipoid-pore concept of the membrane as originally advanced by Collander and Bärlund is still tenable if it is supplemented by the concepts of active transport and facilitated diffusion.

The evidence for the existence of pores in the cell membranes or surfaces is of two kinds: (1) The dependence of diffusion rates on molecular diameter (Collander *et al.*, Pappenheimer *et al.*, Solomon *et al.*); and (2) The existence of "solvent drag" on molecules diffusing in an osmotic water stream through the membrane, a phenomenon which requires a continuous water phase (Ussing; Koefoed Johnsen and Ussing; Andersen and Ussing).

It is pointed out that "sandwich-membranes" consisting of two or more layers with different pore-sizes and different solubility properties may obtain a very high degree of specificity.

The concept of active transport as well as that of facilitated diffusion constitute transport phenomena which cannot be predicted on the basis of the lipoid-pore theory. They differ in that the active transport processes consume metabolic energy whereas the facilitated diffusion processes do not. Kinetically, both types of transport can often be described satisfactorily on the basis of specific membranes carriers, but it is better for the time being to consider the carriers only as abstractions. Despite our lack of detailed information concerning the molecular basis of these processes, the two concepts have proved themselves exceedingly useful in organizing a confusing array of observations into a framework from which predictions can be made.

Finally, it is pointed out that although the phenomenon of pinocytosis may be of importance in special cases, it is extremely unlikely to be among the main vehicles of membrane transport.

4 Biochemical Aspects of Transport. L. E. Hokin and M. R. Hokin (U.S.A.).

Evidence will be reviewed favouring the phosphatidic acid cycle as the mechanism of the Na^+ pump. In this cycle lipid soluble disodium phosphatidate is formed at the inner surface of the membrane from ATP, diglyceride, and 2 Na^+. Disodium phosphatidate crosses the membrane where it is hydrolyzed by phosphatidate phosphatase, releasing 2 Na^+ into the aqueous phase bounding the outer surface of the membrane. Diglyceride returns to the inner surface of the membrane where the cycle is repeated. There is no direct evidence on the mechanism of return of phosphate to the cytoplasm but it is postulated that in those cells which pump Na^+ in exchange for K^+ the phosphate returns with 2 K^+, and in those cells which pump NaCl the phosphate returns in exchange for two Cl^-. The following will be discussed in support of the cycle: (1) the increased renewal of phosphate in phosphatidate on stimulation of the avian salt gland with acetylcholine; (2) the time course of this increased renewal; (3) evidence that the phosphatidic acid effect is in secretory membranes; (4) evidence that the stimulated renewal of phosphate in phosphatidate is catalyzed by diglyceride kinase and phosphatidic acid phosphatase, both situated in the membrane; (5) the requirement of the cycle for Na^+; and (6) the presence of the phosphatidic acid cycle enzymes in the erythrocyte membrane and the evidence that the cycle enzymes may be components of the Na^+ and K^+ dependent membrane ATPase which appears to be closely related to the Na^+ and K^+ pumps.

5 Enzymatic Aspect of Active Transport of Na^+ and K^+ across the Cell Membrane. J. Skou (Denmark).

From peripheral crab nerves a submiscropic particle has been isolated which contains a system fulfilling a number of the requirements for a system involved in the active linked transport of Na^+ and K^+ across the cell membrane.

(1) It contains an enzyme which catalyses the transfer of energy from ATP to the system as an energy-rich phosphate bond.

(2) It has one site where there is an affinity for Na^+ which is higher than for any other monovalent ion and another site where the affinity for K^+ is highest.

(3) The splitting of the high-energy phosphate bond of the phosphorylated compound requires Na^+ in the medium and is highly increased by adding K^+ when the medium contains Na^+, while K^+ alone has practically no effect.

(4) G-strophanthin which specifically inhibits the active linked transport of Na^+ and K^+ in the intact cell also inhibits the $Na^+ + K^+$ activated splitting of the high-energy bond of the phosphorylated compound.

(5) There is indirect evidence that the particle studied is derived from the surface membrane of the intact cell.

(6) A particle which contains a system which has the same specificities for Na^+ and K^+ has also been isolated from other tissues where there is an active linked transport of Na^+ and K^+, namely from brain, kidney and heart muscle.

The relationship of the particulate system to the active linked transport of Na^+ and K^+ across the cell membrane will be discussed.

6 Pharmacological Control of Release of Hormones Including Antidiabetic Drugs. Introductory Remarks. R. COURRIER (France).

7 Pharmacological Actions of the Hypoglycaemic Arylsulphonamides upon the Histophysiology and the Physiology of the Beta-cells of the Islets of Langerhans of the Pancreas. A. LOUBATIERES (France).

The hypoglycaemic arylsulphonamides (thiodiazole or urea derivatives) have a preferential tropism for the pancreas (pancreatotropism) and especially for the beta-cells of the islets of Langerhans (betacytotropism). The betacytotropism is mainly shown by the degranulation of the beta-cells and by other histological modifications demonstrated with electron microscopy.

Hypoglycaemic sulphonamides are betacytotrophic. They produce a hyperplasia of the islets and stimulate the formation of new insulin secretory beta-cells. There follows an increase in the total amount of the beta-cells and an increased capacity of the endocrine pancreas in blood glucose regulation.

We have at the present time strong evidence showing that hypoglycaemic sulphonamides are insulin-secretory stimulating substances. The venous blood coming from the pancreas is more hypoglycaemically active after administration of these drugs that it was before; its "plasma insulin activity" as well as its endogenous insulin content increases. This is the main reason why these drugs are strongly effective in the treatment of human diabetes characterized by sluggishness or relative depression of insulin-secretory process.

The arylsulphonamides manifest an antidiabetic action against meta-alloxanic, meta-hypophyseal or spontaneous diabetes of moderate intensities. They counteract the establishment of idio-hypophyseal diabetes in a dog. They exert a preventive action against the exhaustive process which occurs "spontaneously" in the beta-cells of the pancreas remnant of a subtotally depancreatized animal. These phenomena are in direct connexion with the actions (stimulation, neoformation, regeneration) exerted by these sulphonamides upon the apparatus of the beta-cells. Clinical trials plead for the possibility of the use of these drugs as prophylactic agents against the development of full diabetes in human beings predisposed to this illness.

8 Experimental Investigations with Sulphonureas (S.U.). A. BÄNDER (Germany).

The effects of oral hypoglycaemic compounds upon the enzyme-system, the liver and the peripheral carbohydrate metabolism do not sufficiently explain the hypoglycaemic action. Experimental animals and patients deprived of the pancreas do not exhibit any hypoglycaemic reaction, provided that the gland is removed, for several days.

There is agreement that the main action is to be found in releasing insulin from the pancreas. How this liberation is effected constitutes a question of paramount interest.

According to results by Wrenshall *et al.*, a large number of the diabetics who develop the disease when adults have a sufficient amount of insulin in the gland, sometimes even more than non-diabetics. There remains the question why these patients are diabetics. Some evidence has been obtained that in these patients there is an abnormal binding of insulin in the pancreas. Thus, the difference in blood sugar curves between non-diabetics and diabetics could be explained.

Experiments of Aiman *et al.* showed that plasma-bound insulin could be released by S.U.

Our own investigations have demonstrated *in vitro* a degranulation of isolated β-cells under the influence of S.U.

Reviewing these observations one may assume that S.U. are exerting their effects by means of physico-chemical properties. It is suggested that these qualities produce a removal of this abnormal binding; in this way adequate amounts of insulin are released.

9 Pharmacological Effects of Some Chemical Compounds of Biosynthesis and Secretion of Thyroid Hormones. R. MICHEL (France).

The main steps in the biosynthesis and secretion of thyroid hormones are: iodide concentration, iodotyrosines formation, iodotyrosines coupling, thyroglobulin proteolysis, enzymatic deiodination of iodotyrosines followed by iodothyronines secretion.

Various chemical compounds modify thyroidal iodine metabolism. Thiocyanate, perchlorate and other isoelectric anions of about the same volume inhibit iodide concentration. They act competitively with I^- which probably fits into some specific

sites of oxidase. Thiouracil derivatives not only block iodination but also depress the formation of thyroxine from diiodotyrosine *in vivo* ann *in vitro*. Drugs of this type interfere in some way with thyroxinogenesis and a new iodoprotein appears after a long treatment with propylthiouracil. The relative potency of antithyroid drugs was established by *in vivo* and *in vitro* methods. Relation between chemical structure and antithyroid action was studied. Derivatives of thiourea, mercaptoglyoxaline, thioglyoxalone and mercaptotriazole possess antithyroid properties. The mode of action of these drugs seems to be related to a blockade of oxidizing enzymes involved in organification of iodine. Reducing agents stimulate cathepsins and accelerate the rate of liberation of hormones; thyroxine inhibits deiodination of iodotyrosines preventing uptake of thyroidal iodide.

As iodine metabolism depends of TSH secretion, any compound acting directly or indirectly on the stimulation of TSH produces as effect on hormonogenesis. That is the case for thyroxine, 3:5:3′-triiodothyronine and also for the non-calorigenic 3:3′:5′-triiodothyronine which are concentrated by the pituitary and block TSH secretion. Adrenaline decreases ^{131}I uptake and produces thyroid hyperplasia.

Compounds such as 3:5-diiodo-4-hydroxybenzoylbutylester affect the rate of metabolism of iodohormones; the resulting changes in PBI level modify TSH secretion. Cortisone works by another way, it exaggerates excretion of iodide by kidneys; circulating iodide is reduced and then ^{131}I thyroid uptake decreases.

Thus many chemical products active on peripheral iodine metabolism interfere with biosynthesis and release of thyroid hormones.

10a Action de Quelques Dérivés du 2-Aminomethyl-Benzodioxan sur l'Equilibre Hormonal du Cycle, de la Pseudo Grossesse et de la Grossesse chez la Ratte. F. Bovet-Nitti et G. Bignami (Italy).

Des recherches réalisées sur l'effet de différents dérivés sur la grossesse chez la ratte ont montré que le traitement au cours des premiers jours de la gestation peut provoquer des accidents dont les manifestations n'apparaissent parfois que d'une manière tardive.[1] Dans le cas d'un sympatholytique, le Piperoxan (933F.), l'on a observé que 100 mg/kg administrés par voie orale le jour 1 dé la grossesse empêchent l'ovoimplantation chez 30 à 50 pour cent des rattes sans provoquer d'anomalies chez les autres animaux du lot. L'étude de dérivés voisins montre que cette action ne parait pas liée aux propriétés sympatholyques des ces produits.

Une activité sur la gestation a été également reconnue à certains dérivés du benzodioxan à fonction hydrazine, inhibiteurs des monoamino oxydases (MAO). Dans le cas du N′-1:4-benzodioxan-2-méthyl-hydrazine (2596IS)[2] l'administration de 100 mg/kg par voie orale le jour 1 de la grossesse provoque chez 50 pour cent des rattes soit une inhibition partielle ou totale de l'implantation soit, exceptionnellement, la résorption précoce d'une partie des nidations.

Diverses considérations font penser que l'effet du Piperoxan et du 2596IS est d'prigina centrale et que ces substances sont susceptibles de modifier l'équilibre hormonal par l'intermédiaire des secrétions de l'antéhypophyse. Il a été en effet remarqué[3] que le 2596IS s'oppose dans le 100 pour cent des cas à la pseudogrossesse provoquée par la Réserpine et dans une proportion pouvant aller jusqu'à 40 pour cent à celle dérivant d'une stimulation mécanique.

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1. Bovet-Nitti, F. and Bovet, D. (1959), *Proc. exp. Biol. Med.*, **100,** 555.
2. Bovet-Nitti, F., Orsingher, O., Land-Vittory, R. and Bovet, D. (1961), *Comptes Rendus Acta Scand.*, **252,** 614.
3. Bignami, G., *Rendiconti Ist. Sup. Sanitá.* In press.

10b Action of Certain Derivatives of 2-Amino Methyl-Benzodioxan on the Hormonal Equilibrium of the Reproductive Cycle, Pseudopregnancy and Pregnancy in the Rat. F. Bovet-Nitti and G. Bignami (Italy).

Investigations on the effect of various derivatives on pregnancy in the rat have shown that treatment during the first days of the gestation period can cause accidents which sometimes only become apparent later.[1] In the case of the sympatholytic, Piperoxan (933F) it has been observed that 100 mg/kg administered orally on the first day of pregnancy prevent the implantation of the ovum in 30 to 50 per cent of the rats without causing any abnormalities in the other animals of the group. The study of related derivatives shows that this action does not appear to be related to the sympatholytic properties of these drugs.

Certain derivatives of benzodioxan with hydrazine properties, which inhibit monoamino oxydase (MAO), have also been found to affect gestation. In the case of N′-1:4-benzodioxan-2-methyl-hydrazine (2596IS) the administration of 100 mg/kg orally on the first day of pregnancy provokes either partial or total inhibition of implantation in 60 per cent of the rats or, in exceptional cases, the precocious absorption of the implant.

Various considerations lead to the hypothesis that the effect of Piperoxan and of 2596IS is of central origin and that these substances can modify the hormonal equilibrium through the intermediacy of the anterior hypophyseal secretions. It has, in fact, been observed[3] that 2596IS counteracts the pseudopregnancies due to Reserpine in 100 per cent of the cases and in up to 40 per cent in cases where it is due to mechanical stimulation.

11 Mechanisms by Which Drugs and Hormones Activate and Block Release of Pituitary Gonadotropins. C. H. Sawyer (U.S.A.).

Results of stimulation, lesion and transplantation experiments reveal that the release of pituitary gonadotropins is controlled by the nervous system in both reflexly and spontaneously ovulating animals. Localized electrical stimulation studies have indicated that certain parts of the hypothalamus and rhinencephalon are directly involved. Such drugs as picrotoxin, intraventricular histamine (under weak pentobarbital anaesthesia), copper acetate and intraventricular nor-epinephrine in pituitary-activating dosages stimulate characteristic electroencephalographic (EEG) changes in rhinencephalic pathways, and their influence on the pituitary gland is blocked by basal hypothalamic lesions. Low frequency (5 c/s) electrical stimulation of hypothalamic and rhinencephalic areas evokes a characteristic "EEG after-reaction". Drugs such as atropine, dibenamine and pentobarbital, which block the release of pituitary ovulating hormone, markedly elevate the EEG after-reaction threshold in dosages which do not necessarily elevate the threshold of EEG arousal (evoked by high frequency, 300 c/s, stimulation of the reticular formation). Sex steroids which at first facilitate and later inhibit release of pituitary gonadotropins have temporarily appropriate biphasic effects on the EEG after-reaction threshold whereas steroids which merely block the pituitary only elevate the after-reaction threshold. Antifertility progestogens such as nor-ethynodrel also have a rather specific effect on this threshold. The receptor site of steroid activity would appear to be in the basal hypothalamus as evidenced by ovarian and testicular atrophy following implantation of oestradiol and testosterone, respectively, into the hypothalamus but not following their implantation into other sites in the brain or hypophysis.

12 The Location of Renin in the Rabbit Kidney. W. F. Cook and G. W. Pickering (United Kingdom).

It is not yet exactly known which cells produce renin but the evidence suggests that they occur near the glomeruli. Cook and Pickering (1959) have shown that glomeruli, separated magnetically from rabbit cortex contains a much higher concentration of renin than the remaining non-glomerular tissue. Glomeruli with capsule and attached fragments of closely adjacent tissue contained more renin per mg of nitrogen than glomeruli without these attachments. Glomeruli with attachments can be selected and cut into two halves, one including the vascular pole region and the other not. In this way Cook showed that renin is confined to the vascular pole half of the glomeruli. In this region occur three histologically distinct cell groups, one of which, the juxtaglomerular cells, contain cytoplasmic granules. Others have found that the number of granules in these cells and the renin content of the kidney show parallel changes in several different experimental situations. In view of this it seemed of interest to see whether renin occurred in association with subcellular particles. Rabbit kidney cortex homogenates were first fractionated by differential centrifugation in 0·3 M sucrose. The renin was confined to the "large granule fraction" which was further fractionated on sucrose density gradients. Preliminary experiments show that renin is associated with particles which can be separated from mitochondria. These particles are readily disrupted by mechanical agitation and osmotic shock, whereupon renin is released into solution.

13 The Basophil Response: A New Area in Cytopharmacology. L. Juhlin and W. B. Shelley (U.S.A.).

The chemistry of the circulating basophilic granulocyte (basophil) gives clue to its significant role in the body economy. However, this cell has largely eluded direct observation since it is rare and fragile. Cell counts have been relatively unsatisfactory since they do not regularly reflect the functional activity of the basophil. Recently, we have developed a cytologic technique for observing the morphologic changes in the basophil as it responds to a variety of stimuli. The method consists of rapid cell fixation followed by concentration and selective staining with toluidine blue. The basophils are then classified morphologically (basophil differential) on the basis of the degree of degranulation. The method permits study of the effect of drugs both *in vivo* and *in vitro*.

Results will be presented on the basophil response in man to steroids, histamine liberators, and other pharmacodynamic agents. Particular data will be given on the detection of urticarial and anaphylactoid reactions to drugs.

14 Action of Antihistamine Drugs on Ion and Water Movements in Vitro. K. Ahmed and J. D. Judah (U.S.A.).

Antihistamine drugs have been shown to inhibit water and ion movements in a number of cells and subcellular structures. Mitochondrial swelling is greatly inhibited by these compounds at concentrations at which no effect is seen on respiration or phosphorylation. Mitochondrial contraction is similarly inhibited at the same concentrations. Identical results have been obtained with other systems, e.g. mammalian red cells in which K uptake is inhibited by antihistamines at concentrations which also reduced the rate of osmotic hemolysis. The K uptake of rat liver slices and of Ehrlich ascites tumour cells is also greatly reduced by these drugs.

The mechanism of action of such compounds has been investigated and it is found that phosphoprotein phosphorus turnover is apparently closely

related to ion and water movements in mitochondria liver slices and other systems. It can be shown that compounds which affect ion and water movements greatly reduced the turnover of phosphoprotein-P. In addition, alteration of conditions which result in loss of sodium extrusion (e.g. low K in medium) reduce the rate of phosphoprotein turnover.

The significance of these results for the pharmacological actions of antihistamine drugs will be discussed with special reference to their protective effects against cellular injury both *in vivo* and *in vitro*.

15 Biochemical and Pharmacological Heterogeneity of Mitochondria isolated from Rat Brain. A. S. Moraczewski (U.S.A.).

On the basis of widely scattered evidence, the hypothesis has been formulated that an understanding of drug action on the brain needs to take into account possible differential effects on biochemical activities of different areas of the brain. To test this hypothesis, several phenothiazines were selected and their effects on cytochrome oxidase activity of mitochondria isolated from four gross areas of the brain were studied. It was observed that the levels of enzyme activity differed in the four areas tested and that the order of activity varied with the particular medium used to test the cytochrome oxidase activity. Since the test system involved the rate at which exogenous reduced cytochrome c was re-oxidized, the state of mitochondrial permeability to cytochrome c was an important factor. The final resuspension of mitochondria was done in one of four media: deoxycholate, which solubilizes the cytochrome oxidase system; Triton WR-1339, which fragments the mitochondria; 0·075 M Sucrose, which causes mitochondrial swelling and apparently permits cytochrome c to enter; and 0·25 M Sucrose, which does not favour ready entry of the cytochrome. In addition, the use of these varied media afforded a system which would provide a clue as to whether the drug was exerting an effect on the mitochondrial membrane or intramitochondrially. The data presently in hand suggest that there is some degree of biochemical heterogeneity among brain mitochondria and that there is at least a quantitative difference in the sensitivity to inhibition of cytochrome oxidase activity by phenothiazines.

16 Solubilization and Isolation of Drug-Hydrolysing Enzymes from Microsomes. G. Hollunger and B. Niklasson (Sweden).

Twice washed microsomes from rabbit liver were frozen and thawed about 40 times. The clear, slightly yellow supernatant after centrifugation at 150,000 g for 2 hr contained about one-third of the microsomal protein. Further freezing and thawing did not solubilize more protein. All the iminoacylanilid-hydrolysing activity of the microsomes[1] was solubilized by the procedure. Chromatography of the supernatant on calcium phosphate or TEAE-cellulose columns gave 7 distinct peaks. The chromatographical pattern is quite reproducible. The chromatographical distribution of the iminoacylanilid-, acetanilid-, procaine-, cocaine-, atropine- and acetylcholine-hydrolysing enzymes of the microsomes will be demonstrated. By ultrasonic vibration of the microsomes a soluble protein fraction can be obtained which seems identical with that obtained by freezing and thawing.

A highly active non-dialysable inhibitor of the acetanilid-splitting enzyme was also present in the supernatant after ultracentrifugation. The effect of the inhibitor could be completely removed by small amounts of serum albumen. The purification of the inhibitor will be described.

1. Hollunger, G. (1960), *Acta Pharm. Toxicol. Scand.*, **17,** 374.

17 Activation of Phosphofructokinase from the Liver Fluke Fasciola Hepatica by Serotonin and Cyclic 3:5-AMP. T. E. Mansour, N. A. LeRouge and J. M. Mansour (U.S.A.).

Stimulation of rhythmical movement of intact liver flukes by serotonin resulted in an increased rate of anaerobic glucose uptake and lactic acid production.[1] The stimulation of glycolysis also occurred in homogenates from flukes which had been preincubated with serotonin when glucose, glucose-6-phosphate or fructose-6-phosphate was used as substrate. However, this effect was markedly reduced when fructose diphosphate was the substrate. This indicated that the activity of phosphofructokinase (a rate limiting enzyme in this organism) is increased. Phosphofructokinase activity in homogenates from control flukes was increased by serotonin and cyclic 3:5-AMP (synthesized chemically or by fluke particles). The activating effect of these agents on the enzyme was dependent on the presence of a particulate fraction ATP and Mg^{++} and was inversely proportional to the substrate concentration (Fructose-6-phosphate). When the fluke homogenates were incubated for 20 min at 30°C with ATP and Mg^{++}, a soluble fraction (105,000 × *g*) was isolated which was still activated by the cyclic nucleotide but not by serotonin. Phosphofructokinase in this fraction was further purified by precipitating the enzyme with ammonium sulphate (40 per cent saturation). The enzyme in this precipitate was still activated when preincubated with ATP, Mg^{++} and cyclic 3:5-AMP. Since the production of cyclic 3:5-AMP is increased by serotonin[2] the stimulation of phosphofructokinase by this hormone is possibly mediated via the effect of cyclic 3:5-AMP on a mechanism controlling the activity of this enzyme.

(Supported by N.I.H. Grants E-1386 and SF-512.)

1. Mansour, T. E. (1959), *J. Pharmacol.*, **126,** 212.
2. Mansour *et al.* (1960), *J. Biol. Chem.*, **235,** 466.

18 Mitochondrial Structure as a Controlling Factor of Monoamine-oxidase Activity and the Action of Amine-oxidase Inhibitors. H. Aebi (Switzerland).

The rate limiting factor was studied in suspensions of isolated mitochondria from rat liver, as well as in purified monoamine oxidase (MAO) preparations. Under a variety of experimental conditions the following activities were measured: (1) NH_3-production (Seligson's method); (2) total-O_2-consumption (Warburg's direct method); (3) rate of H_2O_2-production, using the coupled oxidation of [^{14}C] formate by catalase as an indicator reaction; and (4) the aldehyde formation by semicarbazide-trapping.

Mitochondrial suspensions behave quite differently according to their functional state. Compared with lysed mitochondria—as used usually—those suspended in isotonic saccharose and incubated in presence of pyruvate and tyramine exert a much smaller NH_3-production and H_2O_2-formation effect (about $\frac{1}{4}$), whereas their O_2-consumption is 4–5 times higher. Similar differences can be observed if pyruvate is omitted in the suspending medium, if the mitochondrial suspension is frozen before incubation, or if a detergent, i.e. 0·1 per cent Triton-X-100 is added. In all these instances there is reciprocity between the rate of pyruvate oxidation (requiring intact mitochondrial structure) and MAO-activity as well as coupled formate oxidation (either activity being limited by structural factors).

In presence of increasing amounts of MAO-inhibitors all the activities mentioned above are reduced to the same extent, except coupled formate oxidation. Thus, the I_{50}-value of $^{14}CO_2$ formation in isotonic suspensions of rat liver mitochondria is about twice. In presence of *cis*-2-phenyl-cyclopropylamine, added simultaneously with the substrate (0·02 M tyramine) the I_{50}-values for O_2 consumption and NH_3-production are $8{\cdot}0 \times 10^{-6}$ M; for coupled formate oxidation it is $1{\cdot}6 \times 10^{-5}$ M. Furthermore, these I_{50}-values largely depend on the functional state of the suspended mitochondria. In this respect Iproniazid behaves as follows: O_2-consumption and NH_3-production of liver mitochondria, lysed in distilled H_2O, are inhibited 50 per cent by $2{\cdot}5 \times 10^{-4}$ M Iproniazid, on the other hand, an approximate threefold inhibitor concentration (i.e. $7{\cdot}0 \times 10^{-4}$ M) is required if the mitochondrial suspension is incubated under conditions that essentially conserve structural integrity. The significance of these observations is discussed in view of the competitive behaviour of MAO-substrates and inhibitors in mitochondria and in the cell in general.

19 Action of Chlorethoxybutamoxane on Respiratory Enzymes of Rat's Brain in Traumatic Shock. H. Ninomiya, R. W. Buxton and M. Michaelis (U.S.A.).

Several dehydrogenases of rat brain including some which participate in the tricarboxylic acid cycle are depressed in Noble–Collip drum shock (500 turns). The degree of inhibition is in keeping with the severity of the shock, i.e. whether or not the rats' paws were taped or whether they were left unfettered to avoid or diminish impact of the drum. Rats tranquillized with chlorethoxybutamoxane (5-chloro-ethoxy-2-butylamino-methyl, 1-4-benzodioxane) prior to drumming show better resistance to shock. The dehydrogenases of such animals' brain are not significantly changed from controls, or from controls which had not been sedated. There is further differentation between the Pasteur effect in non-sedated shock cases, when repression occurs, and sedated ones, where the effect is lowered but remains at the control level after exposure to drumming. The protein-N contents of brain homogenates of non-sedated and drummed, and sedated, and drummed cases shows statistically highly significant differences.

(Supported with grants from National Institute of Health and from Abbott and Wallace Laboratories respectively.)

20 Drug-induced Alterations in the Intra-cellular Distribution of 5-Hydroxytryptamine in Rat's Brain. N. J. Giarman and S. M. Schanberg (U.S.A.).

Differential centrifugation of rat's brain in isotonic sucrose has shown that approximately 30 per cent of the endogenous 5-hydroxytryptamine (5-HT) is present in the non-particulate fraction, while about 70 per cent resides in granules. A number of neuro-active drugs have been found to alter this "normal" distribution. These drugs fall into two broad categories: (1) those which failed to change, increased, or decreased the level of 5-HT in the whole brain and, simultaneously, tended to shift the particulate to supernatant (P/S; "bound"/"free") ratio from 2·5 toward unity (e.g. chlorpromazine, phenobarbital, reserpine, and α-methyl-DOPA); and (2) those which elevated the whole brain level of 5-HT and tended, at the same time, to maintain a normal P/S ratio or to raise it beyond 2·5 (e.g. iproniazid, phenylisopropylhydrazine, LSD-25, and certain adrenocortical steroids).

(This work was aided by Grant B-940 from National Institute of Neurological Diseases and Blindness.)

21 Modern Concepts in Relationship between Structure and Biological Activity. Introductory Remarks. K. J. Brunings (U.S.A.).

22 Size and Shape of Organic Molecules and Biological Activity. A. H. Beckett (United Kingdom).

Biological processes involve three dimensions. Increasing attention is therefore being focused upon the size, shape and surface characteristics of enzymes, proteins, antigen–antibody reactions, drug–drug receptor interactions. Any chemically mediated, biological action involves interaction between suitably orientated surfaces.

Our knowledge of biological receptors is mainly derived from a knowledge of the size, shape and other characteristics of molecules producing a particular biological response and the characteristics of the activated state are ignored. Examples of accumulation of evidence to help to define several biological receptors are described.

Antibodies may be considered as model biological receptor sites, and hapten–antibody association used to study the importance of size and shape of molecules in the surface interactions. Stereoselective adsorbents prepared from silica may also be used as simple drug–receptor models. Physicoorganic measurements and modern conformational principles facilitate a representation of size and shape of molecules under biological conditions. Effective molecular size may be altered by hydration, aggregation and micelle formation and thus the biological action of a particular type of molecule affected.

Many enzymatic processes are stereo-selective. Penetration of certain membranes is under stereoselective control. The action of many drugs is partly dependent upon their geometry. Side effects of drugs may be largely attributed to the molecules being adsorbed at "undesirable" as well as at "desirable" receptor sites of slightly differing size and shape requirements. One approach to the search for clinically more selective drugs should therefore involve minor modifications on a three dimensional plan of molecules with known biological actions.

23 Chemical Structure, Physical Properties, and Biological Activity. A. Burger (U.S.A.).

This lecture will explore the possibilities that medicinal chemists now have to influence the activity of different chemicals during their paths to their receptor sites and their duration and selectivity of action at these sites. The first problem is to study structural variations or temporary alterations of compounds known to possess a given biological activity if their potency or specificity is inadequate. Protective alterations may keep a reactive compound from being absorbed randomly and prematurely and from being excreted, or they may activate an unreactive molecule so that it will be properly absorbed and distributed. The methods available in these areas will be reviewed.

Analogous considerations are applied to improve the actual reactions or to block alternate metabolic reactions at receptor sites. Following a "lead", the molecule can be changed by increasing or decreasing those binding forces which affect the attachment and maintenance of the drug at the receptor. Apart from the few successful methods of producing irreversible inhibitors by covalent binding, one has to rely on minute and mostly empirical methods of balancing reversible polar, electronic and short-range forces in the molecule. A summary of contemporary work in this field will be given.

Drug activity at receptor sites can also be affected by altering metabolic and blocking reactions. This leads to the intellectually most challenging aspect of medicinal chemistry, the rational design of new biologically active structures. At present, most of the pertinent working hypotheses are still in formative stages, and there are too many exceptions to make them really satisfactory. Theories of metabolite antagonism are overlayed with stereochemical ideas to accommodate various advanced forms of the lock and key fit explanations of drug mechanisms. Since several stereochemical observations have been reviewed by Beckett in the preceding paper, other aspects of reactivity, that is, of various chemical and physical properties, will be dealt with here. How far have we come since the early days of isosterism? How can we get out of the shackles of minor alterations imposed by small molecules as the only acceptable classical procedure? How much should one adhere to biochemical analogy, and how can unorthodox structures be reconciled with metabolite analogues? An attempt will be made to illustrate such cases with established observations as a basis of predictability of novel drug structures.

24 Discussion of Previous Papers. P. Janssen (Belgium) and G. B. Marini-Bettolo (Italy).

25a Inhibiteurs de la Monoaminoxydase et Potentialisation du Sommeil Expérimental. P. Lechat et M. Lemeignan (France).

Les effets sur le sommeil expérimental de l'administration préalable d'inhibiteurs plus ou moins puissants de la mono-amino-oxydase ont été étudiés chez la souris et chez le rat. Les trois hypnotiques utilisés ont été choisis de structure chimique différente: chloral, hexobarbital et méthyl-4-β-chloréthyl-5-thiazole (Hémineurine). Les trois inhibiteurs ont été: isoniazide (100 mg/kg), iproniazide (100 mg/kg) et JL 1314 (10 mg/kg). A ces doses, seuls les deux derniers exercent un pouvoir inhibiteur puissant et sensiblement égal sur la MAO. Ils ont été administrés par voie péritonéale, 30 min avants les hypnotiques, ceux-ci étant injectés par voie veineuse.

En considérant comme seule significative d'une potentialisation, une multiplication par deux au moins de la durée du sommeil témoin, on constate dans ces conditions expérimentales, que: (1) l'hexobarbital est potentialisé d'une façon sensiblement identique par les trois substances chez la souris et chez le rat; (2) le choral n'est potentialisé que par le JL 1314 chez la souris; (3) le méthyl-4-β-chloréthyl-5-thiazole n'est potentialisé que par l'iproniazide chez la souris.

On peut on conclure qu'inhibition de la MAO et augmentation de l'effet des hypnotiques ne vont pas systématiquement de pair, puisque suivant leur nature chimique, les hypnotiques se trouvent ou non potentialisés par des substances dont le pouvoir inhibiteur sur la MAO est très différent.

25b Monoaminoxydase Inhibitors and the Potentiation of Experimental Sleep. P. LECHAT and M. LEMEIGNAN (France).

The effect of preliminary administration of inhibitors of mono-amine-oxydase of varying degrees of effectiveness on experimental sleep was studied on the mouse and on the rat. The three hypnotics used were of different chemical structure: chloral, hexobarbital and methyl-4-β-chlorethyl-5-thiazole (Hemineurine). The three inhibitors were: isoniazide (100 mg/kg), iproniazide (100 mg/kg) and JL 1314 (10 mg/kg). In these doses only the latter two had a marked inhibitory effect on MAO which was approximately equal. They were administered intraperitoneally 30 min before the hypnotics which were injected intravenously.

Considering a duration of at least twice that of control sleep to be a significant sign of potentiation, we found the following results: (1) hexobarbital is potentiated to practically the same extent by all three substances in the mouse and rat; (2) chloral was potentiated by only JL 1314 in the mouse; (3) methyl-4-β-chlorethyl-5-thiazole was only potentiated by iproniazid in the mouse.

It can be concluded that the inhibition of MAO and the augmentation of the effect of hypnotics do not systematically go hand in hand as, depending on their chemical composition, the hypnotics are either potentiated or not by substances which show very different potency as MAO inhibitors.

26 Further Studies on Monoamine Oxidase Inhibitors. S. S. PARMAR and M. NICKERSON (Canada).

We previously reported that several hydrazine derivatives, including iproniazid (Marsilid) and pheneprazine (Catron), react relatively irreversibly with the active sites on the enzyme monoamine oxidase (MAO) to produce a "non-equilibrium antagonism". In the present study the antagonism between hydrazine MAO inhibitors and amphetamine and two of its derivatives (P-1726, *p*-trifluoromethyl, and P-1882, *p*-S-methyl) was investigated using rat liver mitochondria as a source of MAO. Enzyme activity was determined on the basis of both oxygen uptake and substrate (tyramine) utilization, which were found to give equivalent results. Preincubation for 15 min with subinhibitory concentrations of amphetamine or of P-1882 protected MAO against inactivation by pheneprazine. Greater protection by higher, inhibitory, concentrations of these agents was demonstrated by determinations of enzyme activity after dialysis. With respect to both inhibition of MAO and protection against pheneprazine inhibition, D-amphetamine was more active than the L-isomer. P-1726 was found to be a more potent and considerably more persistent inhibitor of MAO than either amphetamine or P-1882. It was not possible to demonstrate antagonism between pheneprazine and P-1726. However, the action of the latter was antagonized by amphetamine. The above results indicate that amphetamine, P-1726, P-1882, and pheneprazine all combine with the same active sites on the MAO molecule.

27 A New Test for MAOI Detecting Effects. A. LEHMANN and R. G. BUSNEL (France).

Specific effects of substances related to the metabolism of biogenic amines led us to study the effect of monoamine oxidase inhibitors (MAOI).

The clinical anti-depressing effect of these substances can hardly be studied on animals or only indirectly by using their antagonism against reserpine.

As reserpine potentializes lethal audiogenic convulsions in most animals an antagonism between reserpine and MAOI can be demonstrated. The test is quite clear: (a) total protection (suppression of seizure); (b) partial protection (clonic seizure only or tonic non-lethal seizures); and (c) death after convulsions.

The experimental drugs are: Tranylcypramine, nialamide, iproniazide, harmaline and feeble MAOI's like benactysine and D-amphetamine. Injections intraperitoneally to mice of the sub-line Rb.

Only tranylcypramine (30 mg/kg), nialamide (500 mg/kg), benactysine (80 mg/kg), and D-amphetamine (40 mg/kg) give a 100 per cent protection against audiogenic seizures.

On the other hand, all these drugs injected at threshold, non-protective doses (tranylcypramine (25 mg/kg), nialamide (20 mg/kg), iproniazide (100 mg/kg), harmaline (30 mg/kg), benactysine (60 mg/kg), D-amphetamine (10 mg/kg) and associated to reserpine (1 mg/kg) prevent the mortality during seizure generally provoked by reserpine alone and give complete or partial protection.

This antagonism is quite different from that obtained when associating tranquilizing drug like carbamate of methyl pentynol, and reserpine; in this case one only obtains a protection with a dose of tranquilizer high enough to have a protecting action by itself (50 mg/kg). It seems worthwhile to draw attention to this test for detecting MAOI effects. One objection can be raised: imipramine (25 mg/kg) and azetazolamid (50 mg/kg) have a similar action as the classical MAOI's but at present we have no biochemical proof that they act like MAOI's. This must still be investigated.

28 Depletion of Catecholamines by Shigella Shigae Toxin in the Mouse Brain. K. MAŠEK, R. SMETANA and H. RAŠKOVÁ (Czechoslovakia).

The influence of *Shigella shigae* toxin on the content of serotonin, adrenaline and noradrenaline in mouse brain was investigated.

In preliminary experiments the content of these compounds in the brain was measured every 12 hr. It was found that maximal changes develop 48 hr

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after the toxin administration. On an average, the content of serotonin and noradrenaline in pooled brains was about 50 per cent of that of the controls. *Shigella shigae* thus causes a considerable depletion of catecholamines in the brain.

29 Excretion of Vanilmandelic Acid by Psychiatric Patients as Related to Drug Therapy. I. Munkvad and A. Randrup (Denmark).

The demonstration that several psychopharmaca affect brain amines, seems to furnish a clue for further studies on the modes of action of these drugs. Measurement of the influence of the drugs upon the excretion of the amines and their metabolites in urine may thus be of interest, as this could give some information on the direct or indirect influence of the drugs upon the production and the ways of metabolism of the amines. As it is also possible to measure the urinary excretion products in the clinical situation, we have chosen this procedure for studying the mode of action of some drugs in psychiatric patients.

In earlier publications from this hospital the effect of reserpine and of iproniazid upon adrenaline and noradrenaline excretion was reported. This work has now been extended by measurements of vanilmandelic acid, the oxidized excretion product of these two amines. The vanilmandelic acid is isolated by high voltage electrophoresis at pH 3 and measured colorimetrically. The effects of chlorpromazine (in varying doses), reserpine and tetrabenazine is studied.

30 Suppression by Iproniazide of the Antagonistic Action of Reserpine on Amphetamine "Group Toxicity". B. N. Halpern and C. Baracco-Drudi (France).

Reserpine is known to reduce the "group toxicity" of amphetamine in mice, as does chlorpromazine. It has been found in this laboratory that treatment with iproniazide suppresses the effect of reserpine and raises the amphetamine "group toxicity" in reserpine-treated animals to the level of the controls.

The time interval between the injection of iproniazide and reserpine is an essential factor. In our experiments, iproniazide is injected first, followed by the injection of reserpine, while DL benzedrine is administered, in all cases, 4 hr after reserpine. No inversion of the action of reserpine is observed, if the interval iproniazide–reserpine is less than 4 hr. The inversion is regularly observed during the time interval 4–36 hr.

The effect of chlorpromazine is not affected by pretreatment with iproniazide. The mechanism of the inversion by iproniazide of the action of reserpine on the "group toxicity" of amphetamine in relation with the cerebral metabolism of aromatic amines will be discussed.

31 Studies on the Mechanism of Uptake of Catecholamines by Isolated Brain Tissues. E. O. Titus and H. J. Dengler (U.S.A.).

Norepinephrine-^{3}H (NE) in slices of cat cerebral cortex incubated with 5 mμg/ml of the labelled amine in Krebs bicarbonate approaches a steady state concentration approximately 4 times that in the medium after 45–60 min. The uptake mechanism, which operates against a concentration gradient and becomes saturated at levels near 100 mμg/ml may be an active transport. It does not function at 0°C and like active transport of 5-hydroxytryptamine in platelets is inhibited by reserpine and cardiac glycosides. Since administration of 3 mg/kg of phenyl α-methylprophylhydrazine (JB 516) 24 hr before the experiment or preincubation of brain slices in 10^{-6} M JB 516 is without effect on uptake, this amine oxidase inhibitor is used to minimize NE breakdown during transport studies. After 45 min incubations, 70 per cent of the isotope in the medium is NE (determined chromotographically). Isotope emerging from the slice in the steady state is accounted for by the following percentages: NE, 42; epinephrine, 2; normetanephrine 35; acidic products of amine oxidase, 19. Corresponding percentages from slices without JB 516 are: 13·6, 10·7, 6·2 and 70·2, respectively. Since 80 to 90 per cent of endogenous NE does not exchange with isotopic NE in 2 hr, NE taken up by the transport mechanism must equilibrate very slowly or not at all with the stored NE in isolated slices. The data suggest existence of at least two intracellular pools of NE.

32a Teneur en Nor-Adrenaline du Tissu Cerebral d'Animaux soumis a l'action de l'Aminodipropionitrile. M. Beauvallet et J. Fugazza (France).

En 1952, Delay *et al.* ont constaté que l'aminodipropionitrile:$HN = (CH_2\text{-}CH_2\text{-}CN)_2$ (I.D.P.N.) provoque chez la souris une agitation motrice permanente: l'animal présente une activité généralisée avec forte tendance à tourner en rond.

Les rats soumis à l'injection du même produit réagissent de façon voisine, présentant des troubles de la coordination motrice avec perte d'équilibre.

Dans ce travail, nous avons recherché la teneur en nor-adrénaline du tissu cérébral du rat et de la souris avant et après l'injection d'I.D.P.N.

Les premières expériences ont été faites sur des rats de race Wistar de 60–100 g; 3 groupes de 6 femelles et 1 groupe de 6 mâles reçoivent deux injections intrapéritonéales d'I.D.P.N. à 48 heures d'intervalle. Le tissu cérébral est prélevé dès l'apparition du syndrome excito-moteur. En même temps on prélève le cerveau d'un animal normal de même portée, de même sexe et de même poids.

Les résultats que nous avons obtenus montrent que la teneur en nor-adrénaline du tissu cérébral du rat mâle ou femelle soumis à l'action de l'I.D.P.N. est très voisine de celle de l'animal normal.

Nous avons trouvé des taux parfois légèrement inférieurs, parfois légèrement supérieurs. C'est ainsi que chez les femelles normales la plus grande quantité de nor-adrénaline observée a été de 0·23 μg/g, la plus faible étant de 0·18; chez les femelles injectées, le maximum est de 0·25 et le minimum de 0·16. La moyenne des différentes valeurs obtenues est de 0·22 μg/g chez les rats normaux et de 0·20 μg/g chez les animaux soumis à l'action de l'I.D.P.N.

Une deuxième série d'expériences a été entreprise sur la souris. Comme chez le rat, nous n'avons observé que de faibles variations—augmentation ou baisse—de la teneur en nor-adrénaline du tissu cérébral de la souris tournante.

Les résultats que nous avons obtenus montrent donc que la nor-adrénaline du tissu cérébral ne subit pas de variations importantes lorsque le syndrome excito-moteur se constitue.

32b Nor-Adrenalin Content of the Cerebral Tissue of Animals under the Influence of Aminodipropionitril. M. Beauvallet and J. Fugazza.

In 1952 Delay *et al*. found that aminodipropionitril:$HN=(CH_2\text{-}CH_2\text{-}CN)_2$ (IDPN) causes permanent motor agitation in the mouse; the animal shows generalized activity and a strong tendency to turn in circles.

Rats, submitted to injections of this substance react in a similar way; they show disturbances of motor co-ordination and loss of equilibrium.

In the present investigation we studied the nor-adrenalin content of the brain tissues of rats and mice before and after the injection of I.D.P.N.

The first experiments were performed on rats of the Wistar strain, weighing 60–100 g; 3 groups of 6 females and 1 group of 6 males received 2 intraperitoneal injections of I.D.P.N. at intervals of 48 hr.

The cerebral tissue was removed as soon as the motor-excitation syndrome appeared. At the same time the brain of a normal animal of the same litter, sex and weight was removed.

The results obtained by us show that the nor-adrenalin level in the cerebral tissue of the male or female rat, subjected to the action of I.D.P.N., is similar to that of normal animals. The values obtained were either slightly lower or slightly higher. Thus, the largest amount of nor-adrenalin found in normal females was 0·23 μg/g, the lowest was 0·18; in injected females the maximum was 0·25 and the minimum 0·16. The mean of the different values obtained in normal rats was 0·22 μg/g and in animals which had been given I.D.P.N. it was 0·20 μg/g.

A second series of experiments was carried out with mice. As in rats, only slight variations (increases or decreases) in the nor-adrenalin content of the brain tissues of revolving mice was found.

The results obtained by us show that nor-adrenalin in the cerebral tissues does not undergo any important variations while the motor-excitation syndrome develops.

33 The Effect of Eserine on the Activity of Adrenergic Nerves in the Rat. V. Varagić, R. Lešić, J. Vuco and B. Stamenović (Yugoslavia).

It has been repeatedly found that eserine raises the blood pressure of the rat anaesthetized by urethane (Varagić, 1955; Dirnhuber and Collumbine, 1955; Hornykiewicz and Kobinger, 1956) as well as of the conscious rat (Medaković and Varagić, 1957). Several factors influencing this effect of eserine have been studied. Pretreatment with reserpine regularly abolished the hypertensive response to eserine. The slow intravenous infusion of noradrenaline, L-DOPA and 5-hydroxytryptamine restored the hypertensive effect of eserine only occasionally. Bretylium and choline 2:6-xylyl ether bromide significantly depressed or abolished the hypertensive effect of eserine. Cocaine was found to antagonize the action of bretylium. In doses which depressed the action of eserine bretylium did not inhibit the hypertension due to excitation of medullary centres induced by clamping the common carotid arteries. Pretreatment with *iso*propylisonizid did not antagonize the inhibitory action of reserpine on the hypertensive response to eserine.

Similar results were obtained by recording the electrical activity in the sympathetic fibres in the mid-cervical region. It is concluded that the available evidence indicates that the hypertensive effect of eserine in the rat is due to central activation of adrenergic nervous elements. Liberation of nor-adrenaline (and adrenaline) from the adrenals and from the blood vessels by eserine does not play a significant role in causing the hypertensive effect of eserine.

34 The Relation between Structure and Central Nervous Action of some Hydrazine Derivatives. A. Spinks and E. H. P. Young (United Kingdom).

About 300 derivatives of hydrazine have been prepared and examined by several biological tests for activity on the central nervous system and particularly for their ability to cause hyperactivity in mice subsequently injected with reserpine. The compounds tested included both straight and branched chain aralkylhydrazines and their acyl derivatives, bis-aralkylhydrazines and aryloxy-alkylhydrazines. The effect of these structural changes, and especially of nuclear substitution, on biological activity has been studied and some relationships have been observed.

It was found that the most promising compound in respect of activity and low toxicity was α-methylbenzylhydrazine (α-phenylethylhydrazine). Nuclear substitution in this and other classes usually reduced activity, and 3:4-dichloro-substitution of many

N_1-aralkyl-N_8-acyl hydrazines caused the appearance of powerful sedative, instead of anti-depressant activity.

35 Mode of Action of Antifibrillatory Drugs. E. M. Vaughan Williams and L. Szekeres (United Kingdom).

Investigators still disagree how antifibrillatory drugs act. The problem is how they prolong the effective refractory period (minimum interval between stimuli for propagation of second action-potential) although they do not affect the absolute refractory period. Some still believe increased duration of action potentials responsible, others that antifibrillatory drugs slow down the recovery of mechanism by which depolarization is achieved.

To test these two hypotheses, measurements were made, on the same preparations of isolated rabbit atria, of: (a) highest frequency stimuli could be followed; (b) threshold current for producing fibrillatory responses; (c) conduction velocity; (d) contractions; and (e) intracellular potentials, during exposure to drugs of dissimiliar structure, quinidine, procaine, procaine-amide, papaverine and dibenamine, from 5×10^{-6}–3×10^{-5}.

All drugs reduced maximum driving frequency and increased fibrillation threshold, dose–response curves being parallel. All reduced conduction velocity, but hardly affected contractions at the concentrations used. The time taken for repolarization to half the full resting potential was unchanged, a fact consistent with absence of change in absolute refractory period. The time for complete repolarization was either unchanged, or slightly prolonged at high doses to an extent insufficient to account for the great prolongation of effective refractory period. The resting potential was unchanged.

In contrast, the rate of rise of the action potential was greatly reduced by all drugs, implying that the property held in common was interference with the phase of depolarization. Such interference would necessitate a longer recovery period before the depolarization mechanism could be reactivated, and so would prolong the effective refractory period.

36 Competition between β-Haloalkylamines and Norepinephrine for Sites in Cardiac Muscle. R. F. Furchgott and S. J. Kirpekar (U.S.A.).

Four β-haloalkylamines, *N*-α-napthylmethyl-*N*-ethyl-β-bromoethylamine (SY28), *N*-cyclohexylmethyl-*N*-ethyl-β-chloroethylamine (GD31), phenoxybenzamine (PB), and Dibenamine (DB), have been studied on isolated, left atria of guinea pigs. These agents do not antagonize the cardiac stimulating effects of catecholamines, and therefore it is concluded that they do not react with cardiac β-receptors. However, they do produce certain effects, which are listed below along with postulated mechanisms of action: (1) Prolonged positive inotropic effect and depletion of endogenous norepinephrine (NE) in normal atria. Mechanism: reaction with storage and binding sites for NE. Potency: SY28 > GD131 > PB > DB; (2) Transient positive inotropic effect on atria from reserpinized animals after "repletion" of such atria with NE, followed by irreversible blockade against further "repletion". Mechanism: reaction with binding sites for NE, probably on nerve terminals. Potency: SY28 > GD131 > PB > DB; (3) Marked irreversible potentiation of inotropic effect of added NE. Mechanism: reaction with sites with which NE combines in the process of its active removal or inactivation. Potency: PB > DB > GD131 > SY28.

All of these agents irreversibly block adrenergic α- (excitatory) receptors of smooth muscle (potency: SY28 > PB > DB > GD131). The sites with which they irreversibly compete with NE in heart may have some reactive grouping in common with smooth muscle α-receptor sites. However, the varying orders of potency associated with the different activities may indicate fundamental differences in the nature of the sites under consideration.

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37 Theophylline Structure as Related to Cardiac Inotropic Activity. H. F. Hardman (U.S.A.).

The relationship between changes in chemical structure and pharmacological activity is complex. Albert[1] emphasized the importance of drug ionization as a factor to be considered in such relationships.

The methylated xanthines such as theophylline and caffeine have measurable positive inotropic activity on cardiac tissue of several animal species. In the pH range of 6·5–8·5 theophylline ionizes as an anion (p*K*a 8·6) whereas caffeine lacks an ionizable hydrogen and cannot exist in the anionic state. Both drugs exist primarily in the non-ionized state at pH 7·4 suggesting that their cardiac inotropic action may be related to this physical form of the drugs. Halogen substituted derivatives of theophylline which exist primarily in the anionic state at pH 7·4 lack positive inotropic activity.

The isolated perfused turtle heart (*Chrysemys picta*) was employed to evaluate the effect of drug ionization upon cardiac inotropic activity in a series of methylated xanthines having different ionization characteristics. This preparation was chosen because pH per se over the range of 6·5–8·5 does not affect the rate or amplitude of contraction of the preparation. This situation allows one to examine the relative effect of the ionized and nonionized form of the drug.

The conclusion reached was that cardiac positive inotropic activity in the methylated xanthines was directly related to the concentration of nonionized drug presented to the turtle heart. Confirmatory evidence was obtained in mammalian cardiac tissue within a more restricted pH range.

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1. Albert A. (1954), *Pharm. Rev.*

38 Mechanism of Cardiovascular Actions of Heptanolamines. J. Garrett and W. Oswald (Portugal).

On the basis of experimental results chiefly obtained on the papillary muscle preparation, heptaminol (6-amino-2-methyl-2-heptanol hydrochloride; 2831 RP) and methylheptaminol (6-methylamino-2-methyl-2-heptanol hydrochloride; 3738 RP; Aranthol) were proposed for clinical use as myocardial stimulants with cardiotonic actions similar to those of cardiac glycosides. However, as these aliphatic amines show definite sympathomimetic effects, it is necessary to elucidate the mechanism of their actions on the heart, in order to determine if digitalis-like properties are involved in these effects.

Tests used in the present study included blood pressure and nictitating membrane of the cat, guinea pig and rabbit isolated atria, isolated rabbit ear, ECG of the cat and the dog, ballistocardiogram of the dog, isolated seminal vesicles of the guinea pig, and rabbit intestine. The effects of heptaminol and methylheptaminol on these tests were compared with those of suitable drugs (catecholamines, tyramine, k-strophantin), the influence of atropine, cocaine and reserpine being also investigated.

The results of the present experiments show that both heptanolamines have a long-lasting cardiostimulant action, which is abolished by cocaine and absent in reserpine-treated animals. The pharmacological activity of these drugs may be entirely attributed to an indirect sympathomimetic action of the tyramine type, possibly due to the liberation of endogenous catecholamines. None of the experimental findings is consistent with the alleged digitalis-like action of these compounds.

39a Substances Hypotensives dans des Extraits Trichloracetiques de Coeurs de Lapins. J. Jacob et L. Maitre (France).

Des extraits trichloracétiques de coeurs de lapins ont été purifiés et fractionnés avec les méthodes, légèrement modifiées, de Von Euler *et al.*[1] (adsorption sur alumine) et de Crawford *et al.*[2] et Vogt[3] (chromatographie sur papier, milieu phénol-HCl).

Ils contiennent une (ou des) substances(s) thermostable(s), abondante(s) surtout dans les éluats de la bande voisine de la ligne de départ mais migrant de façon diffuse, et qui provoque(nt) de l'hypotension chez le rat non démédullé et de la bradycardie sur l'oreillette isolée de rat. Il ne s'agit ni de dérivés de la choline, ni d'histamine, ni de 5-hydroxytryptamine, ni d'une amine catéchique hypotensive. L'effet tensionnel resemble, sans lui être identique, à celui de l'adénosine; il est réduit par l'hexamethonium.

En outre, dans 9 cas sur 11, les éluats des bandes correspondant au RF de l'isopropylnoradrénaline ont eu des effets hypotenseurs et (ou) inotropes positifs (en opposition aux éluats des bandes correspondant à l'adrénaline qui étaient hypertenseurs et moins actifs sur l'oreillette isolée).

Les activités ont varié, correspondant à 0·5–11 mg d'isopropylnoradrénaline par gramme de coeur (soit 0·05 à 1·2 pour cent des amines catéchiques totales). Toutefois ces valeurs sont incertaines: l'isopropylnoradrénaline est très labile à ces concentrations, et les équivalents déduits des pouvoirs hypotenseurs étaient nettement supérieurs à ceux trouvés dans les essais sur l'oreillette; ceci plaide contre l'identification du principe actif à l'isopropylnoradrénaline ou tout au moins contre l'obtention de cette dernière sans contaminants.

1. Von Euler and Orwen (1955), *Acta Physiol. Scand.*, **33,** Suppl. 118, 1.
2. Crawford and Outschoorn (1951), *Brit. J. Pharmacol.*, **6,** 8.
3. Vogt (1952), *Brit. J. Pharmacol.*, **7,** 325.

39b Hypotensive Substances in Trichloracetic Acid Extracts of the Rabbit Heart. J. Jacob and L. Maitre (France).

Trichloracetic acid extracts of rabbit heart were purified and fractionated by slightly modified methods described by von Euler *et al.*[1] (adsorption on aluminium), by Crawford *et al.*[2] and by Vogt[3] (paper chromatography with phenol-HCl).

They contain one or more substances which are thermostable and abundant, particularly in the eluate of the band next to the origin, but migrate diffusely and provoke hypotension in the non-medullated rat and bradycardia in the isolated rat-auricle. The substance (or substances) in question is not a derivative of choline or histamine nor of 5-hydroxytryptamine nor of a catecholic hypotensive amine. The effect on blood pressure resembles that of adenosine without being identical with it; it is reduced by hexamethonium.

In addition, in 9 of 11 cases, the eluates of bands which correspond to the RF value of isopropylnoradrenaline had hypotensive effects and (or) positive inotropic effects (in contrast with eluates of the bands which correspond to adrenaline which were hypertensive and less active on the isolated auricle).

The activities varied; they corresponded to 0·5–11 mg of isopropylnoradrenaline per gram of heart (or 0·05 to 1·2 per cent of the total catecholamines). However, these values are uncertain: isopropyl-noradrenaline is very labile in these concentrations, and the equivalents which were arrived at from hypotensive potency were definitely higher than those found in tests on the auricle. This indicates that the active principle is not identical with isopropyl-noradrenaline or at least that it has not been obtained uncontaminated.

40 Action of Leptodactyline on the Isolated Vagus-Auricle Preparation of the Guinea Pig. A. N. Corbascio and K. Greeff (U.S.A. and Germany).

Leptodactyline (*m*-hydroxyphenethyl) trimethyl ammonium, is a naturally occurring quaternary ammonium base recently isolated from Leptodactylus by Erspamer. Since he has shown it to possess marked nicotinic actions and neuromuscular blocking properties, its effects were studied on the isolated vagus–auricle preparation of the guinea pig and in the rat phrenic–diaphragm preparation. The findings were as follows: (1) Leptodactyline at concentrations of 1–10 μg/ml induces a marked negative inotropic effect on the contractions of the isolated guinea-pig auricle, suspended in Tyrode solution at 28°C. (2) This response is abolished by atropine (0·1 μg/ml). After atropinization, larger doses of leptodactyline (10–25 μg/ml) induced a 50 to 75 per cent increase in amplitude and a 10 per cent increase in the rate of contraction of the auricular muscle. This response was blocked by large doses of hexamethonium (100–200 μg/ml). (3) The threshold to vagal stimulation of the auricle was not appreciably changed by the presence of leptodactyline in the bath (1–50 μg/ml). (4) Total neuromuscular blockade was observed on the isolated phrenic–diaphragm preparation of the rat only after very large doses of leptodactyline (50–500 μg/ml). It therefore appears, under these circumstances, that the nicotinic effects of leptodactyline are more readily elicited than its neuromuscular blocking actions.

41a Uber die Foerderung der Energiegewinnung am erschoepften Herzmuskel durch ein Pyrimidinderivat. W. Kunz und M. Siess (Germany).

Strophanthin führt am überlasteten Herzvorhof des Meerschweinchens nach positiv inotroper Wirkung zu diastolischem Erschöpfungsstillstand, der mit einem Schwund der energiereichen Phosphate um etwa 30 Prozent verbunden ist. Dabei findet eine Na^+-Aufnahme und eine K^+-Abgabe in der Muskelfaser statt. Eine erneute Ansprechbarkeit auf positiv inotrop wirkende Stoffe wie Adrenalin oder Strophanthin findet sich erst nach einer Erholungszeit von etwa einer Stunde, in der das $\sim PO_4$ wieder aufgebaut und die Na^+- K^+-Verhältnisse wieder normalisiert werden. Diese Erholungszeit wird durch 2:6-Bis (diäthanolamino)-4:8-dipiperidino-pyrimido-5:4-D-pyrimidin (Persantin, Thomae) (P.) um etwa 75 prozent verkürzt unter Beschleunigung der Resynthese der $\sim PO_4$. P. verlängert die positiv inotrope Wirkung von Strophanthin am überlasteten Vorhof.

Auch der durch Anaerobiose hervorgerufene Energiemangelstillstand wird durch P. unter Restitution des $\sim PO_4$ aufgehoben. Adrenalin und Strophanthin wirken im Gegensatz zu reiner Anoxie bei P.-Anwesenheit sogar noch positiv inotrop.

Die biochemische Untersuchung ergab, dass eine Anreicherung von DPNH, welche durch Anaerobiose und gleichzeitige Ausschaltung der wasserstoffverbrauchenden Brenztraubensäurehydrierung mittels NaF erzeugt wurde, durch P beseitigt wird. Die Analyse mit ^{14}C markierter Glucose (C_1 oder C_6) ergibt eine Beschleunigung der C_6-Dekarboxylierung bei unveränderter Geschwindigkeit des Glukose-6-Phosphatshunts. In Verbindung mit Metabolitanalysen und Untersuchungen der Atmung und Glykolyse ergibt sich der Schluss, dass die Übertragung des aus dem Tricarbonsäurecyclus stammenden Wasserstoffs durch P als Wasserstoffakzeptor unter Energiegewinnung katalysiert wird.

41b Energy Gains by the Exhausted Heart Muscle with the Aid of a Pyrimidine Derivative. W. Kunz and M. Siess (Germany)

Strophanthin causes diastolic exhaustion-arrest of the overloaded guinea-pig atrium following a positive inotropic effect; this is due to the loss of approx. 30 per cent of the energy-rich phosphates. There is an uptake of Na^+ and a loss of K^+ by the muscle fibres. Renewed reactivity to positively inotropic substances such as adrenalin or Strophanthin only occurs after a recovery period of approximately 1 hour during which the $\sim PO_4$ is again built up and the Na^+ and K^+ relationship has returned to normal. This recovery period is shortened by about 75 per cent by 2:6-bis (diethanol amino)-4:8-dipiperidino-pyrimido-5:4-D-pyrimidin (Persantin, Thomae) (P) with acceleration of $\sim PO_4$ resynthesis. P prolongs the positively inotropic effect of Strophanthin on the overloaded atrium.

Arrest, due to lack of energy caused by anaerobiosis is also abolished by P, through the restitution of $\sim PO_4$. Adrenalin and Strophanthin, in contrast with pure anoxia, have an even positive inotropic effect in the presence of P. The biochemical investigation showed that the accumulation of DPNH, which was caused by anaerobiosis and the simultaneous inhibition of the hydrogen-accepting pyruvic acid by means of NaF, was eliminated by P. Analysis by means of ^{14}C, labelled glucose (C_1 or C_6) results in acceleration of C_6-decarboxylation without change in the rate of the glucose-6-phosphate transfer. The conclusion reached, after combined analysis of metabolites and investigation of respiration and glycolysis, was that the transfer of hydrogen derived from the tricarboxylic acid cycle is catalysed by means of P which acts as a hydrogen-acceptor with a gain of energy.

42 Mechanism of Action of Histamine on the Heart. M. F. Murnaghan (Canada).

Isolated guinea-pig auricles were accelerated with a continuous infusion of histamine and the amount of veratramine require to cause a 50 per cent inhibition of acceleration, i.e. I_{50} dose, deter-

mined. As the minimal rate to which the auricles could be brought with veratramine was less than the initial rate, the I_{50} dose could assume two values depending upon whether the initial or minimal rate was used in calculating acceleration and its percentage inhibition. The terms "corrected" and "uncorrected" refer to the use of the minimal and initial rates respectively in the calculation. The I_{50} dose against histamine was 0·33 μM/l. "uncorrected" or 1·23 μM/l. "corrected". Reiter[1] accelerated isolated guinea-pig auricles with a continuous infusion of adrenaline and obtained an I_{50} value of 0·04 μM/l. "uncorrected" or 0·15 μM/l. "corrected" (the latter value calculated by this author). Evidently, veratramine is approximately 18 times more potent as an anti-accelerator agent against adrenaline than against histamine.

Innes *et al.*[2] have suggested that veratramine exerts two distinct effects, the one that of competitive antagonism towards the accelerator effect of sympathomimetic amines, the other being an independent less sensitive negative chronotropic action. This latter action probably accounts for its antagonism towards the cardio-accelerator effect of histamine.

The results indicate that the chronotropic effect of histamine is due to a direct effect on the sino-auricular node and is not due to catecholamines liberated by the histamine from a storage site in in the heart. Mannaioni[3] has recently reached a similar conclusion from a study of the effect of histamine on auricles treated with reserpine and dichloro*iso*proterenol.

1. Reiter, M. (1956), *Arch. exp. Path. Pharma.*, **229,** 233.
2. Innes, I. R. and Krayer, O. (1958), *J. Pharmacol.*, **124,** 245.
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43a Activite Pharmacodynamique des Aspartates sur le Coeur Isole. M. Lamarche et M. Tapin (France).

Nous avons étudié l'activité pharmacodynamique sur le coeur isolé des aspartates de magnesium et de potassium (composés racemiques) ainsi que des mélanges des deux. Les coeurs utilisés, prélevés rapidement après saignée de cobayes adultes, etaient maintenus en survie selon la technique de Langendorf et perfusés avec le liquide de Chenoweth et Koelle oxygéné.

Dans ces conditions experimentales, l'addition à une concentration de l'ordre de 10 pour cent au liquide de perfusion d'un des espartates utilisés entraine une nette augmentation de l'amplitude de la contraction du coeur isolé (20–30 pour cent), sans que le rythme cardiaque ne presente de variation notable, sauf pour les concentrations élevées du sel de potassium. Cette action se maintient pendant la durée de la perfusion avec le liquide contenant le produit, mais cesse aussitôt que celui-ci est remplacé par le liquide ordinaire, l'experience étant ainsi reproductible à volonté Enfin, il est à noter que cette action nette sur l'amplitude des contractions, sans modification du rythme ne s'accompagne d'aucune variation du débit du coeur isolé.

Nous avons ensuite recherché l'activité des aspartates sur le comportement du coeur lors d'une anoxie. Celle-ci était réalisée dans nos experiences par le remplacement de notre solution de perfusion oxygénée par une autre non aérée. Dans ces conditions, on observe une diminution progressive de l'amplitude des contractions, qui peut être reversible si l'experience n'est pas trop prolongée. Ces essais nous ont montré que la presence d'aspartate dans le liquide de perfusion retardait notablement l'apparition de ces manifestations d'anoxie cardiaque.

43b The Pharmacodynamic Activity of Aspartates on the Isolated Heart. M. Lamarche and M. Tapin (France).

The pharmacodynamic activity of magnesium and potassium aspartates (racemic compounds) as well as of a mixture of both was studied on the isolated heart. The hearts used were removed soon after adult guinea-pigs had been bled and were kept alive by the Langendorf technique and perfused with Chenoweth and Koelle's fluid which has been oxygenated.

Under these experimental conditions the addition of one of the aspartates added in a concentration of about 10 per cent to the perfusion liquid caused a definite increase in the amplitude of contraction of the isolated heart (20–30 per cent) without causing any marked variation in the cardiac rhythm; except with high concentrations of potassium. This effect continued during the time of perfusion with the fluid containing the substance but stopped as soon as it was replaced by ordinary fluid; the experiment could thus be repeated at will. Finally, it must be noted that this definite action on the amplitude of contractions, which did not affect the rhythm, was not accompanied by any variation in the output of the isolated heart.

Subsequently, the activity of aspartates on the behaviour of the heart during anoxia was studied by replacing an oxygenated perfusion fluid by another, non-aerated one. Under these conditions a progressive decrease of the amplitude of contraction was observed which was reversible if the experiment was not too prolonged. These trials have shown that the presence of aspartate in the perfusion fluid markedly delayed the appearance of anoxic cardiac manifestations.

44 The Relationship between Potassium and the Action of Digoxin in the Guinea Pig. G. A. Stewart (United Kingdom).

Slow intravenous infusion of non-toxic doses of

digoxin into guinea pigs increases the K content of the ventricles and serum. Toxic doses produce a further rise in serum K whereas the amount of K in the ventricles does not differ significantly from that of untreated animals.

Intravenous infusion into guinea pigs of KCl solution (8·4–134 mM) simultaneously with digoxin (56 μg/ml) fails to influence the lethal dose of digoxin, but with 268 mM solution of the salt the lethal dose of digoxin is decreased ten-fold.

The time of onset of the positive inotropic response of the isolated right ventricle of the guinea pig, stimulated electrically, to a toxic dose of digoxin is not influenced when the KCl concentration of the bathing fluid is raised from 5·6 to 13·5 mM.

The rate of increase of the amplitude of contraction is markedly reduced but the maximum attained is unaltered and the life of the ventricle is greatly prolonged.

The duration of the therapeutic action of digoxin in the failing heart-lung preparation of the guinea pig is increased if the K content of the circulating fluid is increased from 5·6 to 6·1 mM, but considerably decreased when it is raised to 12·1 mM.

These data suggest that: (1) the extracardiac tissues are more sensitive than the myocardium to the action of digoxin on K flux. and (2) that K, depending on its concentration in the heart, can increase or decrease the cardiotoxicity of digoxin.

45 Pharmacology of Chlordiazepoxide (Librium) and Analogues. L. O. Randall and B. Kappell (U.S.A.).

Chlordiazepoxide (Librium) is the first member of a new chemical class of compounds which has shown unique taming effects in animals[1] and powerful anti-anxiety effects in human subjects.[2] Other pharmacological effects first observed in animals and later confirmed in human subjects include anticonvulsant activity and muscle relaxant effects at low doses, with sedative effects and appetite-stimulating effects at high doses. Certain pharmacological effects which were minimal in animal tests were also minimal in human subjects including effects on blood pressure, heart rate and autonomic nervous system.

Analogues of chlordiazepoxide (7-chloro-2-methylamino-5-phenyl-3H-1:4-benzodiazepine 4-oxide hydrochloride) showed a similar type of activity in muscle relaxant and anticonvulsant tests. Screening tests include the inclined screen test in mice, the fighting mouse test,[3] the anti-strychnine test and the gross behaviour test in cats. Benzodiazepines having activity of interest include 7-chloro-1-methyl-5-phenyl-3H-1:4-benzodiazepin-2(1H)-one; 7-chloro-2-oxo-5-phenyl-1:2-dihydro-3H-1:4-benzodiazepine 4-oxide; 5-phenyl-7-trifluoro-methyl-3H-1:4-benzodiazepin-2(1H)-one; 7-chloro-2-oxo-5-phenyl-1:2-dihydro-3H-1:4-benzodiazepine; 7-chloro-5-(2-chlorophenyl)-3H-1:4-benzodiazepin-2(1H)-one and 7-nitro-5-phenyl-3H-1:4-benzodiazepin-2(1H)-one.

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3. Tedeschi, R. C., Tedeschi, D. H., Mucha, A., Cook, L., Mattis, P. A. and Fellows, C. J. (1958), *J. Pharmacol.*, **125,** 28.

46 The Effects of Ethanol and Chlordiazepoxide in Altering Autonomic Responses Evoked by Isocortical and Paleocortical Stimulation. M. N. Carroll, jr., E. C. Hoff, J. F. Kell, jr. and C. G. Suter (U.S.A.).

Unipolar stimulation of the anterior sigmoid gyrus (I), limbic lobe (II), hypothalamus (III), N. Von Bechterew (IV), amygdala (V), and central gray (VI) loci in flaxedilized cats evoked pressor responses, cardiac arrhythmias (nodal and ventricular extrasystoles and ventricular tachycardia with indications of coronary insufficiency), mydriasis and striking inhibition of mobility of the pyloric sphincter and ileum. Salivation was elicited from subcortical loci II, III, IV and VI, and retraction of the nictitating membrane was noted from I, III and VI. Lacrimation was not observed from any of the areas studied.

The intravenous administration of chlordiazepoxide (10–20 mg/kg) elicited a transient hypotention and slight bradycardia whereas 10 per cent ethanol (3 ml/kg, i.v.) produced increased pyloric mobility and slight to marked alterations in the electrocardiogram. Chlordiazepoxide greatly attenuated pressor responses from all brain areas studied whereas ethanol exerted weak anti-pressor effects in areas I, II and strong blocking action against central stimulation of III. Centrally-induced cardiac arrhythmias were completely blocked by chlordiazepoxide in loci II and V whereas ethanol partially attenuated the response only from II. Neither compound showed any activity against centrally evoked salivation, mydriasis or gastrointestinal inhibition.

Inasmuch as chlordiazepoxide lacks the undesirable side effects of ethanol, its prophylactic potential against centrally-induced arrhythmias and pressor responses merits intensive investigation.

47 Effects of Chlordiazapoxide (Librium) and Other Psychopharmacological Agents on "Fixated" Behaviour in Rats. W. T. Liberson, A. Kafka and E. Schwartz (U.S.A.).

Effects of various therapies were investigated on fixation rats[1] with regard to: (1) motor coordination; (2) sensory and symbolic discrimination and speed of their acquisition; (3) reaction times to stimuli during approach, avoidance or escape behaviour; (4) variability of behaviour; (5) compulsive rigidity; and (6) variety of patterns

of learning. While forced guidance may, none of the tested drugs or electric shock therapy has "cured" rats once fixated behaviour became established. At high doses (1 mg/kg) LSD_{25} blocks goal-directed behaviour. Chlorpromazine, reserpine and meprobamate suppress avoidance and reduce escape. In higher dosages, they also slow down learning and depress approach behaviour. Before establishment of fixation, i.e. during the training period, high doses of tranquilizers prevent fixation in 20–35 per cent of cases. Barbiturates in doses sufficient to affect avoidance interfere with motor behaviour. MOA inhibitors and related agents (iproniazid, amphetamine, Tofranil and Catron) quicken the approach reactions and, in high doses, increase the rate of avoidance behaviour, but do not significantly affect fixation. Chlordiazapoxide (Librium) differs from previous drugs. Although it induces docility, it shortens reaction time of both avoidance and approach to a greater degree than MAO inhibitors. The behaviour becomes very variable, and Librium given during training period prevents fixation in 50 per cent of the rats. Moreover, Librium fosters learning and the effects of guidance and reduces latency times, while most of these were inhibited by chlorpromazine. Thus, although Librium affects fixation prophylactically in greater percentage of cases than other drugs employed, it does not cure established fixation.

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48 On Mechanism of the Antidepressant Action of Imipramine. F. Sulser and B. B. Brodie (U.S.A.).

The potent antidepressant effect of the iminodibenzyl derivative imipramine is clinically established. Pharmacologically, the drug exerts weak chlorpromazine-like actions in normal animals and men. None of the screening procedures currently used would suggest this drug to be an antidepressant drug. The antidepressant action of imipramine becomes dramatically evident in studies which show that it prevents in rats and dogs the depression (chronic pretreatment) and increased central parasympathetic activity (single dose) evoked by reserpine or a synthetic benzoquinolizine (RO-4 1284). However, imipramine does not prevent the action of chlorpromazine. Studies of the physiological disposition of imipramine indicate that the drug acts through a metabolic product, monomethyl norimipramine. This compound is much more active in blocking reserpine or RO-4 1284; furthermore, it shows, in contrast to imipramine, no delay in its action. It not only prevents the action of reserpine or RO-4 1284 but actually reverses it, the animals displaying considerable hyperactivity. Neither imipramine nor its metabolite block monoamine oxidase; they do not increase levels of brain serotonin and norepinephrine or prevent reserpine and RO-4 1284 from releasing brain amines. The possibility that imipramine or its metabolite reveal their antidepressant action by blocking free serotonin will be discussed.

49 Unusual Central Depressant Properties of a New Piperidine Compound. T. B. O'Dell, M. D. Napoli and J. H. Mirsky (U.S.A.).

One approach to the study of the complexities of the central nervous system and its functions is through the use of new pharmacodynamic tools. Such tools might be developed by the investigation of properties of new compounds having novel or unusual chemical structures. Analysis of the responses obtained with novel compounds, coupled with known actions of standard drugs, could well provide new leads toward a better understanding of the functioning of the central nervous system.

A series of new piperidine derivatives has been investigated using a battery of standard pharmacological testing procedures. An unusual combination of effects on the central nervous system has been revealed from these studies. One compound, in particular, 4-(3-indolylethyl)-1-phenethylpiperidine hydrochloride demonstrated strong analgesic activity comparable to that found with morphine, and could also produce a tranquilizing effect which resembled that produced by reserpine. These effects could be separated dose-wise with analgesic activity being apparent at doses below those required to induce the tranquilization responses. True sedative effects (i.e. sleep) could not be induced regardless of the magnitude of the dose administered. The duration of these responses, especially the tranquilizing action, was very prolonged and was a function of the dose employed. Thus, compared to the actions on the central nervous system of known drugs, this new compound appears to possess a unique combination of properties.

50 Influence of a New Group of Tranquillizers, Derivatives of Dibenzosuberene, on the Central Nervous System. Z. Votava, J. Metyšová and M. Soušková (Czechoslovakia).

In the group of 16 new dibenzosuberane and dibenzosuberene analogues of chlorprothixene, interesting correlations between the chemical structure and pharmacological effect on the central nervous system were found. Tests on prolongation of thiopental anaesthesia, rotating rod, depression of the body temperatures, anti-pentazol action and tentative conditioned reflexes revealed that the double bond in the middle ring of dibenzosuberane increased the tranquillizing activity. Further rise of the activity was produced by the introduction of chlorine in the position two of the cycle. The most promising drugs of this type were chloroheptadiene and chloroheptatriene.

51 Pharmacological Action of a New Antidepressant Drug, Propazepine, in Comparison with Imipramine. J. METYŠOVÁ and Z. VOTAVA (Czechoslovakia).

In the search for new drugs with antihistaminic effect carried out in the year 1956, a series of homopromazine derivatives was synthetized. One of these drugs, N-(3-di-methylaminopropyl)-homoacridane hydrochloride, is a structural analogue of imipramine. The pharmacological action of this drug, under the name propazepine, was tested in comparison with imipramine.

Both drugs show similar acute toxicity and action in the test of the rotating rod. Contrary to chloropromazine they do not lower body temperature and their effect on the vegetative nervous system is low.

Both drugs do not affect substantially the toxicity of the central nervous stimulants, amphetamine, pentazol and strychnine. On the other hand, an interesting difference in the interaction with the toxicity of caffein was found. Propazepine decreased the toxicity of caffeine, whereas imipramine and especially chloropromazine increased the toxicity.

52a Mode de l'Action de la Centrophénoxine et ces Dérivatives chez le Poisson. H. NAKAJIMA, J. R. L'HUILLIER, L. BAJINSKI et J. THUILLIER (France).

La Centrophénoxine (ester diméthylaminoéthylique de l'acide *p*-chlorophénoxyacétique) provoque chez le poisson une excitation pouvant entrainer des convulsions et une dilation des chromatophores. Cette action est étudiée en comparaison avec d'autres drogues neuro et psychotropes. L'influence du pH et les conditions physico-chimiques de la réaction sont examinées. L'antagonisme entre Mélatonine et Centrophénoxine est spécialement analysé.

52b Mode of Action of Centrophen-oxin and Derivatives in Fish (Behaviour and Chromatophores). H. NAKAJIMA, J. R. L'HUILLIER, L. BAJINSKI and J. THUILLIER (France).

Centrophenoxin (dimethylaminoethyl ester of *p*-chlorophenoxyacetic acid) induces excitation in fishes capable of producing convulsions and dilation of the chromatophores. This action has been compared with other neuro- and psycho-tropic drugs. The effects of the pH and physico-chemical conditions of the reaction have been studied. A special analysis has been made of the antagonism between Melatonin and Centrophenoxin.

53a Analyse Pharmacologique de la Narcose et de la Stimulation Respiratoire Provoquées Simultanément par une Même Drogue. J. THUILLIER (France).

Le diethylamide de l'acide 2-méthoxy-4-allyl phénoxyacétique (G. 29.505) est le premier composé chimique connu possédant à la fois des actions narcotiques et analeptiques respiratoires puissantes.[1] L'anesthésie générale obtenue avec cette substance est profonde et de courte durée. En même temps que la narcose, l'injection intraveineuse de G.29.505 provoque une stimulation respiratoire plus forte que celle obtenue avec la plupart des analeptiques connu; cette action eupnéique peut s'opposer à la dépression respiratoire provoquée par la morphine ou des composés analogues.

Des hypothèses sont discutées pour tenter d'expliquer la coexistence dans une même molécule de deux actions thériquement opposées: narcotique et analeptique.

53b Pharmacological Analysis of Concomitant Narcosis and Respiratory Stimulation Induced by a Single Drug. J. THUILLIER (France).

The diethylamide of 2-methoxy-4-allylphenoxyacetic acid (G. 29.505) is the first chemical compound known to possess at the same time narcotic and powerful analeptic respiratory actions.[1] The general anaesthesia obtained with this substance is deep and of short duration. Together with narcosis, intravenous injection of G. 29.505 causes stronger respiratory stimulation than obtained with most known analeptics: this eupneic action can resist the respiratory depression induced by morphine or similar compounds.

There is a discussion of hypotheses attempting to explain the co-existence in the same molecule of two theoretically opposed actions: narcotic and analeptic.

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54 The Pneumokinetic Activity of Dimefline (3-Methyl-7-Methoxy-8-dimethylamino-methylflavone, Rec 7-0267) compared with that of Other Central Nervous System Stimulants. I. SETNIKAR (Italy).

Dimefline has been compared with the following drugs for pneumokinetic activity: (a) other brain stem stimulants (bemegride, picrotoxin, pentetrazol and 2:3-dimethyl-7-methoxy-8-morpholino-methylchromone or Rec 7-0105); (b) some other general central nervous system stimulants (prethcamide, nikethamide, diethylamide of 3-ethoxy-4-hydroxybenzoic acid or DEHB); (c) strychnine; and (d) lobeline.

The experiments were performed on non-anaesthetized rabbits. By means of a body plethysmograph the tidal volume (V_T), the pulmonary ventilation (V_{Pul}) and the frequency (F) were recorded.

Pentetrazol, nikethamide, strychnine and lobe-

line caused practically no increase in V_{Pul}. Bemegride, picrotoxin, Rec 7-0105 and DEHB showed a moderate action. Prethcamide and dimefline exerted the greatest and most lasting stimulant action on the respiration, the former acting more on the F and the latter more upon the V_T.

Experiments on rabbits with morphine-depressed respiration yielded similar results. In fact, only dimefline, prethcamide, bemegride, DEHB and picrotoxin brought the V_{Pul} back to normal or supranormal levels.

Taking account of toxicity, the widest therapeutic margin was shown by dimefline, followed by bemegride, prethcamide and Rec 7-0105.

The pneumokinetic activity of dimefline and prethcamide was confirmed in dogs and cats with barbital- or morphine-depressed respiration. Both drugs raised the HbO_2 and the blood pH in addition to the V_{Pul}.

Dimefline is, moreover, highly active in combating respiratory syncope induced by various drugs and in establishing the respiratory function in immature foetuses.

55 Immunosympathectomy as a New Approach to the Study of the Sympthetic System. R. Levi-Montalcini and P. U. Angeletti (U.S.A.).

Adult Swiss mice and albino rats were used. Injections of the antiserum were given soon after birth for 5 consecutive days at the daily doses of 0·05 ml/g of body wt. Histological examination of the superior cervical ganglia was performed in all animals used for the chemical assay of catecholamines and MAO activity.

Adrenaline and noradrenaline were measured in various organs by spectrophotofluorometric procedures. MAO was measured in the same organs by manometric technique. A marked decrease in the noradrenaline content was found in heart, spleen and other tissues of the immunosympathectomized animals. Levels of MAO activity were also found to be decreased in several organs of the same animals. The influence of the administration of various substrates on MAO activity was investigated in normal and experimental mice and rats. The results of the above experiments will be discussed with respect to mono-amine-oxidase activity and sympathetic function.

(Supported by Grant G-13946 from the National Science Foundation.)

1. Levi-Montalcini, R. and Cohen, S. (1960), *Ann. N.Y. Acad. Sci.*, **85**, 324.

56 Cholinesterase Distribution in the Sympathetic Nervous System of the Cat. F. Sjöqvist and B. Fredricsson (Sweden).

Sympathetic ganglion cells are not homogeneous with respect to their content of acetylcholinesterase (AcChE). A small number of the neurons resemble parasympathetic ganglion cells and anterior horn cells in terms of strong histochemical staining intensity for AcChE (thiocholine method). The great majority of the sympathetic ganglion cells have very little, if any, AcChE-activity.

The characteristic AcChE-rich nerve cells are predominantly found in the stellate ganglion and at the level of L6–S1. In these particular ganglia these neurons can amount to 5–15 per cent of the cell population, but in other paravertebral ganglia they are surprisingly few. They seem to be extremely rare in the prevertebral ganglia. Denervation experiments show that most of these cells in the stellate ganglion are connected with nerve fibres to the fore leg.

The results are suggestive of two functionally different types of sympathetic ganglion cells. The AcChE-rich ganglion cells are unevenly distributed in the sympathetic nervous system with an accumulation in those ganglia giving rise to the secretory and vasomotor fibres to the fore and hind foot.[1] This is of interest since the sweat glands of the cat are exclusively found in the paws and are cholinergically innervated.[2] The possible relationship between these AcChE-rich ganglion cells and postganglionic cholinergic sympathetic fibres in general will be discussed.

1. Langley (1891).
2. Dale and Feldberg (1934).

57 On the Mechanism of Action of the Ganglion-blocking Agents. D. A. Kharkevich (U.S.S.R.).

The influence of tetraethylammonium, hexamethonium, hexonium, pendiomide and mecamylamine on the interneuronal transmission of excitation in sympathetic ganglia was studied. It was found that all the drugs tested deepen the passimal (Wedensky) inhibition of the ganglia, increase the time required for transmission of nervous excitation from the pre- to the post-ganglionic fibres (latency) and prevent after-discharges. The influence exerted by ganglion-blocking agents in respect to the above parameters seemed to be fairly uniform. A different result was obtained in experiments with postactivation potentiation. Hexamethonium, pendiomide and mecamylamine decrease or totally prevent the development of potentiation; tetraethylammonium was found to be ineffective.

In view of these data, and considering the nature of postactivation potentiation it may be suggested that hexamethonium, pendiomide and mecamylamine exert a depressing action on the activity of the presynaptic endings.

In order to analyse the action of the ganglion-blocking agents on the conduction of excitation in the post-synaptic neurones, experiments with post-activation inhibition were undertaken (the latter developing as a result of interaction of the hetero-

synaptic volleys, together with recording of the bioelectric activity of the ganglia following antidromic stimulation). It was found that the ganglion-blocking agents do not increase postactivating inhibition and have no influence on the conduction of excitation during antidromic stimulation. In contrast to the ganglion-blocking agents, procaine considerably impairs conduction of excitation in the postsynaptic neurones.

From the above data it appears that the inhibition of transmission of excitation in autonomic ganglia which occurs under the influence of ganglion-blocking drugs is related not only to the selective depression of the cholinoreceptores, but may also be the result of the influence exerted by these substances on the function of the presynaptic endings.

58 Enhanced Postganglionic Responses of Sympathetic Ganglia to Stimulating Agents Following Repetitive Preganglionic Stimulation. R. L. Volle (U.S.A.).

Asynchronous postganglionic discharges of the feline superior cervical ganglion, *in situ*, evoked by acetylcholine (ACh), carbamylcholine, and tetramethylammonium (TMA) were monitored with conventional electrophysiological techniques. Following repetitive preganglionic stimulation with supramaximal volleys at rates ranging from 20 to 100 c/s for periods of 10 sec to 15 min, the amplitudes and time-courses of the ganglionic responses to these agents were enhanced markedly. The duration of stimulation required to produce these changes appeared to be related inversely to the frequency of stimulation. Following repetitive stimulation, carbamylcholine, administered in doses which elicited postganglionic responses lasting 20–30 sec in previously unstimulated ganglia, evoked responses which last 3–5 min. Doses of ACh and carbamylcholine which produced just detectable postganglionic firing were reduced 8- to 16-fold by this procedure of conditioning. The threshold doses of TMA, however, were not reduced; suprathreshold doses were potentiated. The enhanced responses, once induced, persisted for the duration of the experiment (up to 5 hr after conditioning stimulation). Antidromic stimulation of the postganglionic trunks of normal and chronically denervated ganglia did not result in any significant alteration of the responses of the ganglia to these agents. Furthermore, ganglionic blockade by hexamethonium, infused into the arterial supply of the ganglia before and during repetitive stimulation in amounts which blocked completely the postganglionic responses to single preganglionic volleys, did not prevent the occurrence of enhanced responses to injected stimulating agents. It is suggested from these findings that: (1) conditioning with repetitive preganglionic volleys results in alterations in the presynaptic nerve terminals as reported previously by Larrabee *et al.*[1]; and (2) the action of ACh and carbamylcholine on the ganglion is due in part to stimulation of the nerve terminals.[2]

1. Larrabee and Bronk (1947), *J. Neurophysiol.*, **10,** 139.
2. Volle and Koelle (1960), *The Pharmacologist*, **2,** 88.

59 Classification of Ganglionic blocking Agents. J. M. van Rossum (Holland).

Transmission of impulses through the ganglionic synapse is to some extent similar to transmission through the neuromuscular junction. In both cases acetylcholine (ACh) is involved, which implies that drugs may act by: (a) inhibiting the synthesis of ACh; (b) affecting the release of ACh; (c) occupying ACh-receptors whilst having intrinsic activity and thus action as an ACh-mimetic; (d) merely occupying ACh-receptors and thus acting as a competitive antagonist; (e) occupying other receptors and so acting as a non-competitive antagonist.

When studying ganglionic blocking agents on neuro-muscular preparations evidence accumulates that some may act as competitive antagonists and others as non-competitive antagonists.

Ganglionic-stimulants and -blocking agents have therefore been investigated upon the isolated guinea-pig intestine under conditions that only ganglionic effects are measured. Dose–response curves were made of the stimulants using nicotine as a standard, whereas the blockers were studied as how they affect dose–response curves of nicotine.

At least three classes of ganglionic drugs occur: Class I: the mimetics as nicotine, DMPP, pyridine-3-methyl-yl-trimethylammonium; Class II: the lytics or competitive antagonists as hexamethonium, pentapyrrollidinium and azamethonium; Class III: the non-competitive ganglionic blocking agents as pentacyne, chlorisondamine. There exists a number of intermediate compounds, e.g. mecamylamine and pempedine, which are both competitive and non-competitive.

There exists a characteristic relationship between chemical structure and the class of drug.

60 The Effect of Various Amines on Transmission through the Superior Cervical Ganglion of the Cat. S. B. Gertner and A. Romano (U.S.A.).

We have recently reported that guanethidine and bretylium blocked transmission when perfused through the superior cervical ganglion. This block which could be antagonized by the perfusion of epinephrine and norepinephrine, prompted a more thorough investigation of the effects of amines on ganglionic transmission. The actions of epinephrine, norepinephrine, isoproterenol, ephedrine, phenylephrine, tyramine, dopamine, tryptamine, 5-hydroxytryptamine and various other derivatives were tested on transmission. The stability of the

catecholamines during perfusion was greatly increased by the simultaneous use of ascorbic acid, which itself did nothing to transmission. In all the experiments there was never an indication of noticeable stimulation or facilitation of transmission. All of the catecholamines blocked transmission in doses varying from 50 γ/ml upwards. With lower concentrations no effect could be observed. Epinephrine and norepinephrine appeared to be equally effective in blocking transmission; dopamine was considerably less active. Of the drugs tested, tryptamine appeared to be the most potent, often causing considerable block of transmission when perfused in concentrations as low as 10 γ/ml. It was often possible to show a transient block when the drug was given as a single injection of 25 γ into the perfusion stream. Tyramine was somewhat less active. 5-Hydroxytryptamine showed very little if any effect on transmission although sometimes a slight potentiation was noticed. The blocking action of some of the amines was potentiated if given during the perfusion of a mono-amine-oxidase inhibitor.

61 The Mode of Action of Acetylcholine on the Adrenal Medulla. W. W. Douglas and R. P. Rubin (U.S.A.).

It is commonly accepted that the sympathetic nerves innervating the adrenal medulla are cholinergic, and that acetylcholine is the physiological stimulus which effects the release of catecholamines from the adrenal medullary cells. In experiments on perfused cat's adrenals, we have obtained evidence that the action of acetylcholine involves some Ca dependent process. Thus when the adrenals were perfused with Ca-free Locke's solution the amount of catecholamine liberated by acetylcholine fell to 5 per cent or less of the control value obtained in Locke's solution containing 2·2 mM Ca. Moreover, there was a quantitative relationship between the amount of catecholamine released by acetylcholine and the Ca content of the perfusion fluid: catecholamine output was about halved when the Ca concentration was lowered to 0·5 mM, and was increased by about half when the Ca concentration was raised to 8·8 mM. It was found that Ca itself (2·2 mM) caused secretion of catecholamine when introduced after a period of Ca free perfusion: excess Ca (to 35 mM) added during perfusion with Ca containing Locke had no such effect.

These findings, that catecholamine secretion in response to acetylcholine is related to the extracellular Ca concentration and that Ca itself (in appropriate conditions) causes catecholamine release, considered along with the known ability of acetylcholine to effect permeability changes at other post-synaptic sites, lead us to propose that acetylcholine causes catecholamine secretion by promoting an influx of Ca ions into the adrenal medullary cells.

62 The Influence of Ganglion-blocking Agents on Tissue Sulphydryl-Groups Content and Analysis of the Action of Thiol Substances on Impulse Transmission in Autonomic Ganglia. S. A. Mirzoyan (U.S.S.R.).

Some reactive groups of protein bodies undoubtedly play a responsible role in the primary chemical reactions of pharmacological agents with the receptors of effector organs. The present report concerns the interaction of gangliolytics with the tissue sulphydryl groups in structural homogenate. The objects for study have been the superior cervical ganglion and the ganglia of the vagal intestinal fibres in cats. The observations have shown that following the blocking of transmission in ganglionic synapses by hexamethonium, Gangleron or pentamine, a marked decreased in tissue sulphydryl content is observed. The experiments prove that the chemical interaction of gangliolytics with tissue reactive groups is not an accidental or side effect of the examined preparations. In experiments with nicotine it has been shown that the preparation, in doses which augment the sensitivity of ganglionic cells to electrical stimulation of presynaptic fibres and facilitate transmission in interneuronal synapses, causes an increase in tissue thiol groups content. In large doses, the blocking effect of nicotine on ganglionic transmission fully spreads over the sulphydryl groups content.

Titration of the homogenate in the presence of hexamethonium, Gangleron or pentamine, shows that the interaction of these preparations with the tissue sulphydryl groups takes place also in the non-structural homogenate, and under the action of hexamethonium, Gangleron or pentamine, a decrease is noted in sulphydryl groups content.

In perfusion experiments an antagonism is observed between the sulphydryl groups and ganglion-blocking agents in their action on sympathetic ganglia and ganglia of the vagal intestinal fibres.

Thus, gangliolytics exhibit a capacity to decrease the thiol groups content and tissue sulphydryl groups have a definite significance in the chemical sensitivity of receptors towards ganglion-blocking agents.

63 Lipid Solubility as an Important Factor for the Penetration of Drugs into the Liver. H. Kurz (Germany).

The relative rate at which various foreign organic compounds of widely different chemical and physical properties enter the liver tissue was investigated in the isolated rabbit liver. The liver was perfused from the portal vein with a drug–saline solution at constant rate. The perfusate was collected in small fractions over a period up to 5 hr. The rate of disappearance of the drug was calculated from the decrease in concentration in the perfusate. Protein-binding was eliminated by calculation. The experiments were made at a temperature near 0°C to avoid metabolic interference

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and antipyrine was used in all experiments as a reference substance. The experiments showed that compounds with a high oil–water partition coefficient disappeared most rapidly from the perfusate while compounds with low water–oil partition coefficient disappeared at a slow rate. The results thus point to a lipoid barrier between blood and liver tissue which seems to be qualitatively similar to the blood–brain barrier. In a second experiment drug penetration into the liver tissue was studied by using tissue slices. This means that drugs could enter the cells from the incubation fluid without passing through the walls of the blood vessels. In these experiments no important differences in the rate of penetration of the various compounds could be detected. This suggests that the main obstacle for the penetration of drugs is located within or in the close vicinity to the walls of blood vessels.

64 The Inhibitory Effect of Sodium Lauryl Sulphate on the Intestinal Absorption of Glucose and its Potentiation of Phloridzin. J. A. Nissim (United Kingdom).

The inhibitory effects of cationic quaternary ammonium germicides, such as trimethylhexadecylammonium stearate, cetrimide and domiphen, on the intestinal absorption of nutrients have been described previously.[1] Striking enhancement of inhibitory activity on the intestinal absorption of both glucose and sodium butyrate was achieved in cetrimide–phloridzin combinations, which was of the order of 50–100 times for the former and 10–15 times for the latter.[2]

These studies have now been extended to sodium lauryl sulphate. This is an anionic surface-active germicide, which showed no histological injury to the intestinal mucosa when perfused or injected. The compound was perfused into the intestine of pure strain C3H mice with 0·2 per cent glucose in normal saline (25 ml) in half-hour experiments. Values of the percentage absorption of glucose are given in Table 1, and are compared with those obtained with cetrimide.

Sodium lauryl sulphate exhibited a weak inhibitory effect as compared with cetrimide or phloridzin. The potency ratio of lauryl-phloridzin combinations to phloridzin alone, however, was 5·8 with fiducial limits at P = 0·01 of 12·3 and 2·9, while the potency ratio of cetrimide-phloridzin combinations to phloridzin was 16·2 with corresponding fiducial limits of 40·7 and 7·6. Striking reduction of glucose absorption was shown by surfactant-phloridzin combinations at concentrations of the drugs which possessed no inhibitory activity whatever when perfused along (first column). Further, in spite of poor intrinsic activity, sodium lauryl sulphate showed considerable phloridzin-potentiating effect, which was only 1/3γ1/2 that of cetrimide.

Table 1

Percentage absorption of glucose. Figures represent mean of four experiments except where number is indicated in brackets. Control percentage absorption in 27 mice = 35·4 ± 1·1

Substance	Concentration of substance in perfusion fluid					
	0·001%	0·002%	0·01%	0·02%	0·1%	0·5%
Cetrimide	46·6	30·4(10)	24·1		3·7	
Phloridzin	34·3(8)	19·6(8)	10·8(8)		−0·9(8)	
Sodium lauryl sulphate			34·9	28·3	20·8	19·0(3)
Phloridzin[1] +Cetrimide	7·0	5·1	−1·6			
Phloridzin[2] +S.L.S.	16·5	8·4	−0·4			
Phloridzin[1] +S.L.S.			2·3			

(1) Equal conc.
(2) S.L.S. conc. 10 times that of phloridzin.

1. Nissim, J. A. (1960a), *Nature* (*Lond.*), **185,** 222; (1960b), *Nature* (*Lond.*), **187,** 305; (1960c), *Nature*, **187,** 308.
2. Nissim, J. A. (1961). Submitted for publication.

65 Intestinal Absorption Influenced by Calcium-binding Substances. E. Sögnen (Norway).

The presence of calcium-binding substances (Ca-b.s.), EDTA, sodium fluoride and sodium oxalate, in the gastrointestinal tract reduces the effect of orally administered drugs. Thus lethal oral doses of strychnine or barbiturates leave the animals unaffected or only slightly influenced when given concomitantly with Ca-b.s. A decreased plasma level of barbiturate and sulphonamides is found in experimental animals, compared to controls as previously reported.

Further study has revealed that the decreased plasma concentration is not due to increased renal excretion. The influence of Ca-b.s. on factors determining the rate of intestinal absorption has therefore been more closely studied.

Perfusion experiments on isolated small intestine *in situ* and experiments on isolated everted loops of small intestine *in vitro* have shown that the transmucosal translocation of water, glucose and sulphonamides and the utilization of glucose, is reduced by Ca-b.s.

The retard rate of transmucosal translocation is supposed to be caused by the effect of Ca-b.s. on glucose metabolism.

In the intact animal the observed alteration in gastric and intestinal fluid transport results in a dilution of the initially administered solution.

Accordingly, the gastric emptying time of the solute is prolonged. The solution is further diluted during transit through the small intestine. This interrelationship between gastrointestinal water transport, gastric emptying time and rate of intestinal absorption, might be of general importance.

Ca-b.s. do not cause morphological or irreversible functional alterations in the intestine.

The implications of these findings will be discussed in relation to the results of other workers.

66a Gesetzmässigkeiten des Strontium - Stoffwechsels und ihre Bedeutung für die Eliminierung von Strontium aus dem Skelet. A. SCHMID (Deutschland).

Es wird über Gesetzmässigkeiten des Strontium-Stoffwechsels im Skelett, den Umsatzmechanismus *in vivo* und die Fixierung von Strontium im Knochensystem berichtet.

66b Patterns of Strontium Metabolism and their Significance in the Elimination of Strontium from the Skeleton. A. SCHMID (Germany).

The patterns of strontium metabolism in the skeleton, the conversion mechanism *in vivo* and fixing of strontium in the bone system are reported.

67 On the Use of Expiratory $^{14}CO_2$ Patterns as a Pharmacological Tool for Studying the Biochemical Effects of Drugs. G. T. OKITA (U.S.A.).

Since all carbon containing biochemical intermediates are eventually metabolized to CO_2, alteration in their metabolism due to biochemical effects of a drug may be reflected by alteration in the rate at which labelled CO_2 appears in expired air. Therefore, an apparatus which will monitor continuous expiratory $^{14}CO_2$ patterns after the administration of ^{14}C-labelled intermediates is a useful pharmacological tool for studying the mode of action of those drugs having biochemical effects as a basis for their pharmacologic response. An apparatus built in our laboratory for this purpose has been reported elsewhere.[1] Essentially, the instrument consists of a 4 π gas phase Geiger counter, an infra-red gas analyzer for measuring $^{12}CO_2$, a ratio analyzer to compute specific activity ($^{14}CO_2/^{12}CO_2$) of $^{14}CO_2$, and a ventilation meter. All measurements are recorded continously on a 4-channel recorder after the injection of a ^{14}C-labelled intermediate. Depending upon the drug under investigation such labelled intermediates as acetate, pyruvate, lactate, formate, glucose, citrate, etc. have been employed. By the use of appropriate labelled intermediates and comparison of expiration $^{14}CO_2$ patterns between control and drug treated groups, it is possible to obtained information on the biochemical mode of action of drugs.

The effects of testosterone, oestrogen, insulin, orinase and diamox on expiratory $^{14}CO_2$ patterns after the administration of various ^{14}C-labelled intermediates will be presented. Some of the advantages of this method for studying biochemical effects of drugs are: (1) *in vivo* condition, all experiments are conducted on intact, unanaesthetized animals and subjects; (2) simplicity, no individual $^{14}CO_2$ samples to assay; also, 2–6 experiments may be run per day; and (3) utilization of human subjects, therefore, no need to extrapolate animal data.

1. (1960), *Int. J. Appld. Rad. Iso.*, **7**, 273.

68 Metabolic Studies of Carcinogenesis Using Expiratory $^{14}CO_2$ Patterns Following Administration of ^{14}C-Labelled Intermediates. E. A. EZZ and G. T. OKITA (U.S.A.).

Using an instrument developed in our laboratory[1] we were able to measure continuously expiratory $^{14}CO_2$ patterns in experimental animals after the administration of the following ^{14}C-labelled intermediates: acetate-1-^{14}C, sodium bicarbonate-^{14}C, glucose-1-^{14}C and glucose-6-^{14}C. The effect of carcinogenesis and various hormonal states such as ovariectomy, estrogen and testosterone therapy on the expiratory $^{14}CO_2$ specific activity patterns of ^{14}C-labelled intermediates were studied in virgins, exbreeders and mammary tumour C3H mice free of the mammary tumour "milk factor". The specific activity curves as well as ^{14}C levels in expiratory CO_2 showed significant differences in some of the experimental conditions. The most striking biochemical change noted during the carcinogenesis process was the reduction in the percentage recovery of glucose-1-^{14}C/glucose-6-^{14}C. Rank order arrangement for the various experimental groups of C3H mice were as follows: factor free—1·48, virgin—1·36, exbreeders—1·00, tumour (single)—0·84, and tumour (multiple)—0·75. The decrease in the ratio is a reflection of an increase in glycolytic metabolism. Ovariectomy and testosterone therapy to tumour animals tends to return the ratios to those for virgin controls. This supports the thesis that ovariectomy and testosterone therapy tend to correct the metabolic defect produced by tumour.

1. (1960), *Int. J. Appld. Rad. Iso.*, **7**, 273.

69 Studies on the Functions and Mode of Action of Thiamine. C. J. GUBLER (U.S.A.).

Although the symptoms of thiamine deficiency have been well documented, the metabolic disturbances which cause these symptoms are still not well understood. In order to gain a better understanding to these metabolic disturbances, and thus of the physiological functions of thiamine, rats were made

deficient by thiamine-deprivation and by administration of the thiamine antagonists oxythiamine (OTh) and pyrithiamine (PTh). At the onset of symptoms, rats were sacrificed and the oxidative decarboxylation of various α-ketoacids studied in the tissues by a spectrophotometric method with $Fe(CN)_6$ as the electron acceptor. In liver mitochondria thiamine deprivation, OTh-treatment, and PTh-treatment all caused a reduction in the rate of oxidative decarboxylation of pyruvate (to 24·3, 46·2 and 43·1 per cent of the normal rate, respectively). The rate with α-ketoglutarate was affected only by thiamine-deprivation (66·2 per cent of normal). With α-ketoisovalerate and α-keto-β-methylvalerate, none of the deficiencies had a significant effect. In kidney homogenates all three types of deficiency caused a marked reduction in pyruvate oxidation (41·8, 44·3 and 37·9 per cent of normal, respectively) but only thiamine-deprivation and PTh-treatment caused a decrease in α-ketoglutarate oxidation (45·5 and 65·2 per cent, respectively). OTh had no effect. In brain homogenates PTh-treatment caused a decrease in both pyruvate and α-ketoglutarate oxidation (to 48·0 and 50·6 per cent of normal, respectively), while there was no change in thiamine-deprived and OTh-treated brains. The decreased rates of oxidative decarboxylation could be largely restored to normal by the *in vitro* addition of cocarboxylase. Brain and kidney homogenates did not oxidise α-ketoisovalerate and α-keto-β-methylvalerate.

70 Metabolic Pathways of Tetraiodothyro- and Triiodothyro-Acetic and Propionic Acids. E. V. Flock, J. L. Bollman and G. H. C. Stobie (U.S.A.).

Major metabolic pathways for thyroxine (T4) and 3:5:3′ triiodothyronine involve removal of an iodine atom from the benzene ring with the side chain and thus inactivation of these hormones. Conjugates of the products 3:3′:5′ triiodothyronine and 3:3′ diiodothyronine are excreted in the bile of dogs with livers but accumulate in the blood and urine of dogs without livers. Analogues of the thyroid hormones, labelled with ^{131}I in the 3′ or 5′ position, were studied in dogs with biliary fistulas and in dehepatized dogs. In dogs with biliary fistulas tetraiodothyroacetic acid (TETRAC) was metabolized much more slowly than T4; much less ^{131}I was excreted in bile or urine. Large amounts of unchanged TETRAC with smaller amounts of 3:3′:5′ TRIAC were found in the blood. Tetraiodothyropropionic acid (TETPROP) was partially deiodinated to 3:3′:5′ TRIPROP which accumulated in the blood as the amount of unchanged TETPROP decreased. Both of these compounds of propionic acid were excreted in bile as glucosiduronides. Triiodothyroacetic acid and 3:5:3′ triiodothyropropionic acid were rapidly cleared from the blood and excreted in the bile chiefly as glucosiduronides, with small amounts of the sulphoconjugates of 3:3′ diiodothyroacetic acid and 3:3′ diiodothyropropionic acid. In dogs without livers, the sulphoconjugates of these 3:3′ diiodo- compounds were found in larger amounts in both blood and urine, but the major metabolites appeared to be sulphoconjugates of 3′ monoiodo-derivatives.

71 Relations between Structure, Velocity of Biological N-hydroxylation and Toxicity of Aromatic Amines. H. Uehleke (Germany).

N-hydroxylation of aromatic amines in the body forms highly toxic methemoglobin forming hydroxylamine derivatives and seems to be important in allergy and carcinogenesis. Liver microsomes in the presence of TPNH and oxygen can perform the reaction. This is the first time that enzymatic N-hydroxylation has been achieved *in vitro* and that one of the few toxication mechanisms has been elucidated biochemically. The velocity of N-oxydation *in vitro* and *in vivo* depends on the chemical structure of aromatic amines. Aniline, naphthylamine and 2-aminofluorene are slowly oxydized. P-substitution of aniline by electron-attracting groups increases the velocity of N-hydroxylation in the animal and *in vitro*. Compounds with ionizable *p*-substituents (-COOH,-SO_3H) are not measurably attacked, presumably because of fat insolubility. Mono-N-alkylanilines are hydroxylated faster than aniline, the alkyl being removed as aldehyde in the reaction. Levels of oxydized amines in the animal and velocity of N-hydroxylation by liver microsomes are compared with toxicity and methemoglobia forming capacity of the corrresponding amines. The reaction of N-hydroxylation will be discussed in view of other known hydroxylation mechanisms of aromatic compounds. The toxication mechanism described gives us a better understanding of the toxicity and side effects of many drugs.

72 Mechanisms of Activation and Induction of Rat Liver Tryptophan Pyrrolase. O. Greengard and P. Feigelson (U.S.A.).

The parenteral administration of a number of agents cause an increased level of tryptophan pyrrolase activity in the liver of rats. These agents include hormones, drugs and the substrate of the enzyme, tryptophan. The elevation of enzyme activity can be as much as 10-fold.

Tryptophan pyrrolase is an unusual heme-enzyme in that in normal liver much of it exists in the free apo-protein form, the presence of which can be revealed by the addition of hematin to the assay mixture.[1] Increased activity can therefore result both from saturation wth cofactor or from increased apo-protein concentration. Methods have been developed to distinguish between the two mechanisms, and differentiation between the modes of action of the various inducing agents has been achieved. The administration of cortisone or reserpine results in the accumulation of the apo-enzyme form of tryptophan pyrrolase in the liver. However, upon

the administration of tryptophan, allyl*iso*propylacetamide and 3-amino-1:2:4-triazole, saturation with the hematin cofactor occurs, *in vivo*, with a subsequent net increase in enzyme protein, in the form of active holoenzyme.

Studies, both *in vitro* and *in vivo*, showed that the association between apo-tryptophan pyrrolase and hematin is facilitated by tryptophan, suggesting that the concentration of the latter may determine what fraction of the existing apo-protein is catalytically active.

1. Feigelson and Greengard (1961), *J. Biol. Chem.*, **236,** 153.

73 Extraction of a Seizure Substance (K Substance) from the Dog's Brain. T. Hayashi and K. Nagai (Japan).

When an electro-shock current was applied through the skull of dogs, a generalized seizure appeared, which continued 60–180 sec after the cessation of the stimulation. During these seizure, a seizure substance (K substance) was released from the motor cells which rapidly diffused into the cerebrospinal fluid. Fluid thus obtained was introduced into the c.s.f. of another dog; it produced a clonic convulsion in the receiver dog. There were, however, a few difficulties with these experiments, as success did not occur in every case. Recently, Hayashi *et al.* succeeded in all cases, if the fluid was treated with absolute methyl alcohol after reduction of its volume by a third. After centrifugation the fluid was reduced by evaporation in a 40°C water bath to 30 per cent of the original volume. This fluid contained K substance, and when it was introduced into the ventricle of the receiver dog a strong seizure was produced. The K substance could be extracted from the grey matter of the cortex, but not from the white matter.

The active agent was highly unstable in an oxygen atmosphere, but when it was kept in contact with nitrogen the activity was maintained for a long time. Purification and chemical analysis of it is now being carried out in our laboratory.

74 The Physiological Action of K Substance Extracted from Dog's Brain. T. Hayashi and K. Nagai (Japan).

The active agent[1] which was contained in the cerebrospinal fluid of dogs taken during seizure induced by electric stimulation was examined.

When a small amount of it was injected into the exposed motor cortex of a dog, a generalized seizure was produced, as when an electric stimulation was applied. For instance: (a) when concentrated, the clonic convulsion; (b) when diluted, the locomotive convulsion (walking movement); and (c) when more diluted, a tonic movement or twitch of one limb.

When it was introduced into a ventricle, it produced seizure with latent period of 3–20 sec in the receiver dog. When it was rapidly injected through the carotid artery, a seizure appeared with latent period of 2–5 sec.

The agent had no action on the respiration and blood pressure of dogs when injected intravenously. It had no action on an excised toad's intestine, nor on an excised heart preparation.

The action of K substance was neutralized by mixing of an appropriate dose of γ-amino β-hydroxybutyric acid, which we believe was the real inhibitory transmitter of the central nervous system of higher animals. Thus we presume that the agent is the "excitatory transmitter" in the central nervous system of mammals.

1. Hayashi *et al.* "Extraction of a seizure substance (K substance) from dog's brain" in this congress.

75 γ-Aminobutyric Acid and Vagal Respiratory Regulation. J. A. Schneider and A. B. Drakontides (U.S.A.).

γ-aminobutyric acid (GABA), a normal brain constituent, was reported several years ago to have an inhibitory influence on crustacean stretch receptors.[1] Only recently, however, inhibitory or "desensitizing" effects of GABA on slowly adapting pulmonary stretch receptors of the cat were described.[2] Since these receptors are thought to be part of the ascending arc of the Hering–Breuer Reflex, a study of the action of GABA on vagal respiratory reflexes was indicated. Simultaneous recording of whole body plethysmography and discharges from afferent pulmonary stretch receptors in cats were used to analyze respiratory characteristics during GABA injection. Tracheal occlusion responses were utilized to assess vagal respiratory function.

The study indicates that an increase in depth of the inspiratory phase and a transient elevation of the absolute lung volume coincides with the desensitizing effect of GABA on pulmonary stretch receptors. Tracheal occlusion responses, however, were not altered consistently. The relatively short duration of respiratory changes following intravenous GABA administration contrasts markedly with the prolonged inhibitory effect on pulmonary stretch receptors, indicating rapid adjustment of vagal respiratory regulation. The possible physiological significance of GABA in the regulation of respiration remains unclear.

1. (1954), *Arch. Int. Physiol.*, **62,** 33.
2. (1960), *Amer. J. Physiol.*, **199,** 748.

76 On the Activity of γ-amino-butyric-acid Derivatives on Central Nervous System Excitability. A. Bertelli and G. Gavazzi (Italy).

The action of γ-amino-butyric acid and β-hydroxy-γ-amino-butyric acid intraperitonally administered to rats, on the nervous stimulation caused by many exciting and convulsing drugs (strychnine, cardiazol, amphetamine, morphine, NH_4Cl, etc.) was studied.

It was observed that with regard to β-hydroxy-γ-amino-butyric acid and to the greater absorption of the molecule, β-hydroxy-γ-amino-butyric acid exerts a remarkable antagonistic action against the above-mentioned exciting drugs.

77 The Effect of Certain Glutarimides on Brain Metabolism. P. J. Nicholls (United Kingdom).

The convulsants, β-methyl-β-ethyl- and β-methyl-β-*iso*propyl-glutarimides and the hypnotics, β, β-, di-*n* propyl- and α-ethyl- α-phenylglutarimides were examined using rat brain brei. Cholinesterase and amine oxidase were not affected by any of the compounds but there was a slight inhibition of choline-acetylase activity by concentrations of 10^{-3} M of the hypnotic glutarimides. When glucose or glutamate were substrates, the oxygen uptake was inhibited by the convulsant and hypnotic glutarimides in concentrations of 10^{-2} and 10^{-3} M, respectively. This inhibition was reversed by methylene blue. In rat liver mitochondria, concentrations of the drugs inhibiting oxygen uptake had no effect on oxidative phosphorylation. The oxidation of succinate and *p*-phenylenediamine by rat brain was unaffected by the glutarimides. Concentrations of the drugs inhibiting oxidation of lactate caused no appreciable accumulation of co-enzyme neither did they affect the transfer of hydrogen from reduced coenzyme to flavoprotein. With yeast, which does not require coenzyme for oxidation of lactate, the glutarimides inhibited the oxygen uptake in the presence of lactate but they had no action on yeast lactic dehydrogenase.

From these results it is concluded that the glutarimides act at the flavoprotein level. While the convulsant glutarimides are effective only at doses which would be lethal *in vivo*, such an effect may explain the mechanism of action of the hypnotic glutarimides. A similar site of action has been proposed for the oxybarbiturates.[1, 2]

1. Aldridge, W. N. and Parker, V. H. (1960), *Biochem. J.*, **76,** 47.
2. Grieg, M. E. (1946), *J. Pharmacol.*, **87,** 185.

78 The Mechanism of the Antagonistic Action of Reserpine on the Anticonvulsant Effect of an Inhibitor of Carbonic Anhydrase. W. D. Gray, C. E. Rauh and R. W. Shanahan (U.S.A.).

Treatment of mice with reserpine abolishes the anticonvulsant action of methazolamide, an inhibitor of carbonic anhydrase; in contrast, the action of diphenylhydantoin is only slightly affected. Possible modes of action of reserpine are actions on: (1) the absorption and fate of the inhibitor; (2) its penetration into the central nervous system; (3) interference by reserpine on the interaction of the enzyme and the inhibitor; or (4) a functional antagonism, since reserpine is known to enhance the susceptibility of mice to electroshock seizures. Mice treated with reserpine 24 hr before the oral or the intravenous administration of methazolamide showed increased concentrations of inhibitor in plasma, erythrocytes, and in brain. Treatment with reserpine had no action on the localization of carbonic anhydrase and carbonic anhydrase inhibitor following differential centrifugation of homogenates of the brains of mice. Both appear to be localized in the soluble fraction. Three of the suggested possible modes of action do not appear to be involved; the antagonistic action of reserpine possibly operates at a functional (physiological) level or levels within the central nervous system.

79 Tranquilizers and Antidepressants: A Pharmacological Comparison. F. Herr, J. Stewart and M. P. Charest (Canada).

The actions of chlorpromazine and chlorprothixene on a battery of pharmacological tests were compared with those of imipramine in an attempt to discover in animals properties which might correlate with the clinical effects of these drugs. On the basis of these tests, a series of compounds of unknown pharmacological properties was investigated. One of these compounds, amitriptyline, synthesized independently by this and another laboratory, has been studied in detail and is compared with those mentioned above.

The differences in the pharmacological activities of chlorpromazine, chlorprothixene, imipramine, and amitriptyline were quantitative rather than qualitative. All potentiated hexobarbital and alcohol narcosis, decreased body temperature, and caused ataxia. They decreased spontaneous motility, impaired performance of learned responses, and also delayed the learning of these responses. In these tests, in order to obtain a given degree of effect, larger doses were required of imipramine and amitriptyline than of chlorpromazine and chlorprothixene. In spite of the fact that higher doses of imipramine and amitriptyline were required to influence, for example, a conditioned runway response, these effective runway doses were about a fifth of the doses which caused ataxia. On the other hand, the effective runway doses of chlor-

promazine and chlorprothixene were the same as those which caused ataxia.

A suggestion is offered according to which the ratio of effective doses of a compound in different tests may be indicative of its therapeutic effectiveness. Recently, Freed (1960) reported a clinical investigation of amitriptyline which showed its antidepressant properties in depressed patients.

80 Differentiation of Central Depressants by Means of the Veratramine Excitation. W. Schoetensack and G. Hallmann (Germany).

Veratramine in small doses evokes tremors, in higher subletal doses convulsive-like excitation phenomena.

Studies in rats and mice have shown the following results: (1) Tremor and convulsions are only suppressed by a few centrally acting muscle relaxants such as mephenesin, zoxazolamine, 2-aminobenzthiazole, chlormezanone and carisoprodol, whereas meprobamate and 2-(γ-methoxypropylaminomethyl)-1:4-benzodioxane-HCl fail to block the veratramine excitation; (2) A number of unsaturated tertiary alcohols, urethane, chloral hydrate and phenylacetylurea abolish the veratramine convulsions but not the tremor.

Hypnotics and anticonvulsants such as phenobarbital, hexobarbital, phenytoin, and troxidone, also a number of tranquillizers, ganglionic blocking-, sympathicolytic-, and anticholinergic agents are ineffective against tremor and convulsions up to the level of lethal doses.

The two excitation phenomena induced by veratramine differ completely in their unusual resistance against most of the investigated drugs from the excitations produced by leptazol, strychnine or by harmine and tremorine.

Transections of brain in rat suggest that structures within the lower brain stem are responsible for initiating the veratramine excitation.

Transections in the higher brain levels including the diencephalon do not change fundamentally the reactions after application of veratramine. After removal of the cerebellum convulsions appear but not tremor.

Decerebrate rigidity in rat is intensified by the central excitants mentioned above, including harmine and tremorine; veratramine, however, completely prevents the hyperactivity of the extensors and after cessation of the veratramine excitation the decerebrate rigidity appears unchanged.

Since the veratramine excitation is completely inhibited only by a limited number of centrally-acting muscle relaxants, these findings seem to be of considerably value for evaluation and differentiation of central muscle relaxants.

The nervous mechanisms probably responsible for the initiation and evidently specific inhibition of the two stages of the veratramine excitation are discussed.

81 Three Types of Artificially Induced Tremors and the Effect of Some Antiparkinsonian Agents upon Them. I. L. Bonta and H. M. Greven (Holland).

Usually antiparkinsonian drugs are pharmacologically evaluated by testing them against varying types of tremors produced by surgical methods (tremor-monkey) or chemical means (nicotine, Tremorine). In the course of our investigation of the effect of intracerebral drug injections, we observed that a new compound, Gre-1248, induced a type of hyperkinesia (running fits and hind-limb tremor), which reminded us of certain motor disturbances occurring in extrapyramidal syndromes. The effect of nicotine, Tremorine and Gre-1248 on mice will be demonstrated by a short film.

Antagonistical studies with several antiparkinsonian drugs (atropine, caramiphen, trihexyphenidyl, orphenadrine, diethazine, etc.) have shown that the various compounds induced different degrees of protection against the three types of tremors. The question of parallelism between experimentally-induced tremors and Parkinson's disease or related extrapyramidal disorders will be briefly discussed.

82 A Contribution to the Pharmacology of Tremorine-induced Tremor. P. Stern and J. Gašparovic (Yugoslavia).

The onset of tremorine-induced tremor (TT) in mice is delayed by application of iproniazid or PIH 20 hr prior to tremorine (T); similarly applied hydralazine or α-methyl DOPA have the opposite effect. Since iproniazid and PIH increase dopamine, serotonin (S) and GABA in the CNS, the three precursors, DOPA, 5-hydroxytryptophan (5-HTP) and glutamic acid were examined. Only 5-HTP delayed TT and harmine-induced tremor, but not that induced by 3-amino-1:1:3-triphenylpropan-1-ol or diethylcysteamine. DOPA and glutamic acid did not act on either kind of tremor. When T was preceded by iproniazid (20 hr) and 5-HTP (2 hr) TT was delayed considerably further than by action of either substance alone. Consequently, an increase of S in the CNS delays TT, although T itself has no influence upon the S-level. The fact that hydralazine and α-methyl DOPA, two inhibitors of amino-acid decarboxylases, are capable of inducing tremor, favours this assumption. S-antagonists, harmine and LSD also induce tremors. Reserpine and chloropromazine-induced Parkinsonism is interpreted on the basis of these facts. In contrast to chloropromazine, reserpine reduces the level of dopamine, the concentration of which is particularly high in the corpus striatum. The latter contains, in addition, much S, substance P and choline acetylase. We have found that S potentiates this enzyme, but so does T alone. It is open to discussion whether accumulation of S in definite regions, e.g. the corpus striatum, can lead to an inhibition of TT. The present results pre-

clude the possibility of dopamine being the responsible factor.

83 The Blood–Brain Barrier. H. Davson (United Kingdom).

The various factors determining the rate of penetration of substances from blood to the nervous tissue of the brain and spinal cord will be discussed.

84 Exchange between Cerebrospinal Fluid and Blood. J. R. Pappenheimer and S. R. Heisey (U.S.A.).

Large bore cannulae have been implanted over the cerebral ventricles and in the cisternae magnae of goats. Animals survive in good health and their ventriculo-cisternal systems may be perfused with synthetic CSF of normal ionic composition without detectable circulatory, respiratory or behavioural changes. Steady-state transfer rates between CSF and blood can be measured from differences between inflow and outflow of test substances. Normal permeabilities (flux rates per unit concentration difference) to creatinine and inulin average 0·19 and 0·12 ml/min, respectively, and may be accounted for by passive processes. Diodrast and phenol red (in low concentrations) are removed rapidly from CSF to blood by a process of active transport resembling secretion by the proximal tubules of the kidney; clearances of these substances from CSF may exceed 1 ml/min. The locus of active transport occupies a volume of about 2 ml in the region of the fourth ventricle and cistern magna.[1] The extraction of K^+ from CSF perfusion decreases with increasing concentration, suggesting that this ion is also removed by active transport. Preliminary experiments indicate that large increases of K^+ are required to produce a rise in blood pressure comparable with that caused by a small decrease in Ca^{++}. It is possible that an active transport system for K^+ must be saturated before an increased concentration in CSF can penetrate to fluid immediately surrounding neurones.

1. (1960), *Amer. J. Physio.*, **200,** 1.

85 Discussion of Previous Paper. D. P. Rall (U.S.A.).

86 Pharmacology of Aqueous Humour Formation and Outflow. E. Bárány (Sweden).

Some anatomical and physiological facts concerning the ciliary epithelium and the trabecular meshwork in the angle of the anterior chamber will be briefly presented. The methods used for the measurement of rate of production of aqueous humour and its outflow resistance will then be critically surveyed. Finally, what little is known concerning the pharmacology of the secretory system and the outflow system will be discussed.

87 Discussion of Previous Paper. T. Maren (U.S.A.).

88 Pharmacological Control of Prolactin Secretion and Lactation. J. Meites (U.S.A.).

Many substances can induce prolactin release from the anterior pituitary of rats and/or rabbits, and elicit mammary growth and secretion. These include oestrogens, progesterone, testosterone, cortisol, adrenalin, noradrenalin, acetylcholine, serotonin. pilocarpine, eserine, atropine, amphetamine, morphine, reserpine, chlorpromazine, meprobamate and several carcinogens; rat hypothalamic extract and Guillemin's CRF; electrical stimulation of the head, nasal mucosa, nipples, lumbar region and uterine cervix; non-specific stresses such as cold, heat, restraint or formalin injections; and transplantation of the rat pituitary to the kidney capsule. Large doses of oxytocin, vasopressin, histamine, Dibenemine and LSD 25 were unable to elicit mammary secretion.

The effective agents apparently released ACTH as well as prolactin, since both hormones are necessary to elicit mammary secretion in intact rats, and thymus wt. was significantly reduced. These agents were ineffective in hypophysectomized rats. Induction of mammary secretion by pituitary transplantation confirms the view that the CNS normally inhibits prolactin release; however, it is doubtful that all agents used inhibited the CNS, i.e. rat hypothalamic extract, Guillemin's CRF and some drugs. Also, in hypophysectomized rats with pituitary transplants, injections of reserpine, serotonin, adrenalin, acetylcholine or formalin significantly increased the percentage of rats showing mammary growth and secretion. Some agents may therefore stimulate a CNS centre(s) which activates the anterior pituitary to induce mammary growth and secretion. Cultures of pituitary explants *in vitro* secrete substantial amounts of prolactin[1] and studies are now in progress to determine which agents can directly influence this process.

1. Meites, Kahn and Nicoll (1961). Programme of 43rd Meeting, The Endocrine Society, page 3, June 22–24, New York, N.Y.

89 Control of Ovulation in Women. G. Pincus and C.-R. Garcia (U.S.A.).

In women suggestive, but not conclusive evidence of the suppressive action of progestins has been noted by means of the indices of ovulation (basal body temperature, vaginal smears, endometrial biopsies and urinary pregnanediol). Of these, marked reduction of urinary pregnanediol excretion is indeed most suggestive. Furthermore, inspection of the ovaries at time of laparotomy during the latter phases of cyclic administration of progestins also confirms the failure of ovum release. Although inconsistent results as regards reduction of total

urinary gonadotrophin excretion have been reported, it appears that specific individual variation as well as differences in gestagen dosage and duration of administration play important roles. It is most likely that at lower dose levels the cyclic administration of gestagens has a primary effect on luteinizing hormone rather than on the follicle-stimulating hormone. The suppressive action on ovulation is further noted in ovarian biopsies which failed to show developing follicles although there is no reduction in the comparative number of primordial germ cells. The relative delay in the consistent return of regular ovulatory cycles in women so treated after cessation of medication also points to the suppressive nature of this form of therapy. The administration of these gestagens during the post-ovulatory phase in a normal cycle, fails to alter significantly the pregnanediol excretion, and one can safely assume that they do not have a direct, adverse effect on corpus luteum function, and therefore on luteotrophin secretion. Furthermore, the establishment of pregnancy during such form of therapy precludes the possibility of interference with implantation. Although sperm penetrability of endocervical mucus varies during this form of suppression, most patients have a reduced number of spermatozoa present in this area, and this may be a contributory factor to the control of conception.

90 Influence of Certain Neurotropic Agents upon ACTH Secretion. S. V. Anichkov, A. N. Poskalenko and V. E. Ryzhenkov (U.S.S.R.).

The action of neurotropic drugs upon ACTH secretion was estimated by the amount of 17-oxycorticosteroids in the blood (experiments on dogs and guinea-pigs) and ascorbic acid level in the adrenals (experiments on rats). Hypophysectomized animals were used as controls.

Among the neurotropic agents experimented with, alkyl derivatives of diamidimidazolcarboxylic acid (the so-called antifeines) proved to be most powerful in stimulating ACTH secretion.

After a 1–2 mg/kg intravenous injection to dogs the amount of 17-oxycorticosteroids was doubled, and after 10–20 mg/kg intraperitoneally to guinea-pigs it increased four times.

The analysis of antifeine action upon the CNS showed that these drugs exerted a sedative action upon the cerebral cortex, and a stimulating one upon the subcortical formations.

Antifeines are successfully used in neurologic practice. The stimulating action of the antifeines upon ACTH secretion is prevented by amytal, and is not prevented by chloral hydrate and chlorpromazine. Amytal also prevents reflex ACTH hypersecretion.

91 Pharmacological Control of the Secretion of ACTH. P. L. Munson (U.S.A.).

The level of circulating cortisol and/or corticosterone is well established as one important factor in the physiological regulation of ACTH secretion. A second important factor, generally accepted although somewhat controversial, may be the postulated hypothalamic secretory factor for ACTH secretion (HSF-ACTH)—thought to be the mediator in the stress reaction. The study of these two and possibly additional factors in the physiological control of ACTH has advanced but is still far from complete.

A number of aspects of the pharmacological control of ACTH secretion are also of interest and significance. Numerous drugs, especially in high doses, are known to stimulate ACTH secretion. Often classed as non-specific stimuli or stressors and assumed to act on the anterior pituitary indirectly, some of them have also been shown to be effective when applied directly to the gland, both *in vivo* and *in vitro*. Other drugs, notably morphine and some of the tranquilizers, have been shown to depress the rate of ACTH secretion or at least to inhibit the ACTH response to stressful drugs and procedures.

Although the mechanisms of action of both groups of drugs, those that stimulate and those that inhibit ACTH secretion, are still very incompletely understood and therapeutic applications in the control of ACTH secretion seem unlikely, these drugs are useful in experimental work on the nature of the physiological regulation of ACTH secretion. Morphine, for example, by inhibiting the response of the rat to non-specific stimuli such as extraneous constituents of neural tissues, is the basis of promising bio-assay methods for HSF-ACTH in extracts of hypothalamus and posterior pituitary.

92 Pharmacological Alteration of the Growth and Hormone Secretion of Hormone-producing Tumours in Man. R. Hertz (U.S.A.).

Non-hormonal agents which materially alter the metabolic function of hormone-producing tumours may either simply obstruct the biosynthesis of the characteristic hormones or may produce sufficient destruction and regression of tumour mass to materially reduce hormonal output. These effects are also manifested by reduction in the secondary metabolic effects of excessive hormonal production on the organism as a whole.

Examples to be detailed will be: (1) the inhibition of steroid hormonal synthesis by adrenal cancer by amphenone, metuperone and related agents which obstruct specific steps in adrenocortical synthesis; (2) the selective destruction of adrenal cortical tissue and associated tumour regressions induced by a series of agents related to DDD; (3) the total regression and associated hormonal suppression seen in women with tumours of the chorionic trophoblast which are productive of massive amounts of chorionic gonadotrophins.

The problem of the nature of the metabolic change which leads the neoplastic cell of hormone-producing tissue to quantitative and qualitative aberrations in hormone production will be outlined.

93 Methods for the Study of Pharmacological Effects at Cellular and Subcellular Levels. Introductory Remarks. O. H. Lowry (U.S.A.).

94 Use of Histochemistry in Pharmacological Studies. G. B. Koelle (U.S.A.).

The chief obstacle to the localization of sites of drug action at the cellular level is our present ignorance concerning the nature of the groups with which most drugs react to produce their characteristic pharmacological effects, i.e. the receptors. Demonstration of the distribution of a drug does not by itself permit distinction between critical active sites and non-specific storage sites. In a few instances (e.g. anticholinesterase (anti-ChE) agents, carbonic anhydrase inhibitors), the significant tissue component affected is known with reasonable certainty, and in others (e.g. monoamine oxidase inhibitors, mercurial diuretics), potential, but not necessarily the critical, cellular constituents have been identified. When this information is available, histochemical demonstration or microchemical determination of changes in the reactivity of the active group or enzyme produced by the drug may provide more direct information on its site of action. This, of course, does not exclude the possibility of its total pharmacological effect being the result of reaction with more than one type of receptor.

Concrete examples of the foregoing principles are provided by histochemical investigations of the sites of action of anti-ChE agents. Such studies have shown that acetylcholinesterase (AChE) may be oriented externally or internally with respect to the cell membrane, and that certain pharmacological effects are produced by inhibition of only the external AChE. In addition, they have provided evidence of a dual role of acetylcholine (ACh) in synaptic transmission: an action at the presynaptic site, to promote the liberation of more quanta of ACh or of other transmitters, and, in the case of cholinergic neurons, at the postsynaptic site.

95 Contribution of Electronmicroscopy to Neuropharmacological Problems. E. de Robertis (Argentina).

New ultra-structural details of the synaptic region—in addition to the synaptic vesicles[1]—will be described in isolated nerve endings and in sections of the brain cortex. These include a system of intersynaptic filaments and a subsynaptic web forming a tight complex with both synaptic membranes. The possible significance of these two new structures and the physiological role of synaptic vesicles will be discussed.

The so-called "mitochondrial fraction" of the brain has been subdivided into five subfractions, one containing myelin, another only mitochondria, a third containing broken nerve endings and synaptic complexes, and two formed by rather intact nerve endings which represent about 15 per cent of total brain weight.

The AChE and SDH activities and the ACh content of these fractions was determined. The results suggest the probable existence of a different population of cholinergic and non-cholinergic endings in the two fractions. These results also throw some light on the localization of AChE in central synapses.

The ultra-structure of adrenergic nerve fibres and endings with the presence of a specific plurivesicular material will be described. A structurally similar plurivesicular component is found in the secretory processes of the pineal gland. Pharmacological and other experimental studies suggest that this gland is formed by adrenergic neurosecretory cells and that the plurivesicular material is the site of storage of 5-hydroxytryptamine, catecholamines and other biogenic amines secreted by the pineal gland.

These and other results will be interpreted within an unified neurosecretory theory that embodies synaptic processes and neurosecretion.

The contribution of several of our collaborators in these studies will be acknowledged.

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96 Some Morphological and Biochemical Aspects of the Sarcotubular System in Frog Skeletal Muscle Cell. E. Andersson-Cedergren and U. Muscatello (Sweden).

One of the main components of the skeletal muscle cell is a system of tubular and vesicular structures localized in between the myofibrils. From the morphological distribution and characteristic organization of the sarcotubules[1, 2] and from information gained from experiments by local electrical stimulation[3] the conclusion has been drawn that these sarcotubules could be responsible for the propagation of the stimulating impulse from the cell membrane inwards to the contractile material. By means of differential high speed centrifugation this system of sarcotubular elements has been isolated. The isolated fraction from frog muscle homogenate contains small tubular and vesicular elements and small particles not attached to the membranes.[4]

The sedimentation, biochemical and morphological data of the sarcotubular fraction show similarity to the properties of the so-called relaxing factor described by Marsh.[5] The relaxing activity of the sarcotubular fraction is shown by its capacity to inhibit the myofibrillar ATP-ase and the myofibrillar superprecipitation induced by ATP. The activity seems to be localized to the membranous components of the sarcotubular fraction. The fractions of the muscle homogenate containing the mitochondrial fragments or the small particles do not show the same properties.

The biochemical data of the sarcotubular fraction together with the morphological appearance of the

sarcotubular system in the intact muscle cell point to the fact that the sarcotubules possess an ability to regulate the contraction and relaxation of the contractile material.

Some new properties of the sarcotubular system will also be discussed.

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2. Andersson-Cedergren, E. (1959), *J. Ultrastructure Res.*, Suppl. 1.
3. Huxley, A. F. (1957), *J. Physiol.*, **135**, 17p.
4. Muscatello, U., Andersson-Cedergren, E., Azzone, G. F. and von der Decken, A., *J. Biophys. Biochem. Cytol.* In press.
5. Marsh, B. B. (1951), *Nature* (*Lond.*), **167**, 1065.

97 Pharmacological Studies with Isolated Cell Components. V. P. Whittaker (United Kingdom).

Several groups of workers have during the past few years successfully applied the technique of subcellular fractionation to the isolation of cell components concerned with the storage of pharmacologically active substances.

Homogenates of tissues in iso-osmotic sucrose are fractionated by differential centrifugation and by equilibrium centrifugation in a density gradient. The various fractions may be assayed for acetylcholine, pressor amines and other pharmacologically active substances and their morphology studied in the electron microscope. In this way it has been found that the catecholamines, 5-hydroxytryptamine and histamine are associated in adrenal medulla,[1] duodenal mucosa,[2] platelets[3] and mast cells[4] with a dense type of granule (denser than 2·0 M sucrose) about 0·5 μ in diameter which also contains a high concentration of a polyvalent anion (ATP or, in mast cells, heparin). By contrast, in central nervous tissue, acetylcholine, hydroxytryptamine[5, 6] and probably noradrenaline[7] are localized in a fraction which has been identified[8] as consisting mainly of pinched-off nerve endings. Mildly disruptive procedures cause the liberation of the neurohormones and also the disappearance of the synaptic vesicles with which these nerve ending particles are normally packed. The isolation of these different kinds of storage granules provides a new type of preparation on which to study drug action.

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2. Baker (1959), *ibid.*, **145,** 473.
3. Baker *et al.* (1959), *ibid.*, **149,** 55P.
4. Hagen *et al.* (1959), *J. Pharmacol.*, **126,** 91.
5. Hebb and Whittaker (1958), *J. Physiol.*, **142,** 187.
6. Whittaker (1959), *Biochem. J.*, **72,** 694.
7. Chrusciel (1960), *Symposium on Adrenergic Mechanisms.* London: Churchill.
8. Gray and Whittaker (1960), *J. Physiol.*, **153,** 35P.

98 Discussion of the Previous Paper. J. D. Judah (U.S.A.).

99 Effects of Fat-Soluble Compounds on Lysosomes in vitro. C. de Duve, R. Wattiaux and M. Wibo (Belgium).

Lysosomes are special cytoplasmic particles containing a variety of soluble acid hydrolases enclosed within a lipoprotein membrane.[1] They are believed to be involved in phenomena of intracellular digestion and in various forms of physiological and pathological cytolysis. The membrane of the particles may be of fundamental importance in the latter processes, since it normally acts as a protective barrier between the enzymes present inside the particles and a number of vital cell components such as proteins and nucleic acids which are substrates of these enzymes. Agents which influence the properties of the lysosomal membrane may be expected to affect autolytic phenomena in living cells.

The present experiments were initiated as part of a programme designed to explore these possibilities. As test system, the rate of release of bound acid phosphatase in mitochondrial preparations from rat liver incubated at pH 5 and 37° was employed. It has been found so far that testosterone, progesterone, β-oestradiol and deoxycorticosterone all stimulate the release of acid phosphatase under those conditions, when added to the incubation at a concentration of 125 μg/ml. The same effect is obtained with vitamin E which is still active at doses as low as 6·25 μg/ml. On the other hand, cortisone and hydrocortisone (125 μg/ml) exert a small but distinct inhibition of this phenomenon. Further results will be reported.

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100 Analysis of Drug-Receptor Site Interactions. B. Belleau (Canada).

Recent developments pertaining to the mechanism of action of alkylating adrenergic blockers will be summarized. Some chemical properties of receptor sites may tentatively be deduced and a theory based on ion-pairing mechanisms will be shown to account for certain structure–activity relationships amongst catechol amines. Recent work by the author on the central role of monoamine oxidase in the limitations of adrenergic responses will be presented and it will be shown that structure–activity relationships can sometimes reflect monoamine oxidase–substrate relationships rather than receptor site properties. Through the use of deuterium-labelled substrates, it is possible to demonstrate unambiguously that the intensity of an adrenergic response depends mostly on interactions involving monoamine oxidase rather than

adrenergic receptor sites. The stereospecificity of this latter enzyme has been elucidated and some general conclusions regarding its specificity will be presented. The concept of "affinity" and "intrinsic activity" will be examined in the light of these new findings. The mechanism of action of a group of new monoamine oxidase inhibitors will be discussed in relation to the properties of the enzyme receptor sites. Finally, the discovery of a new class of catecholamine analogues allowing for the first time an insight into the role of the catechol nucleus in adrenergic mechanisms will be reported. In this connection, the use of a novel type of potent catechol *O*-methyltransferase inhibitors will be described. Here again, structure–activity relationships will be shown to reflect in some cases the interplay of this latter enzyme rather than the receptor sites themselves.

101 Some Often-neglected Considerations in Structure-Activity Studies. M. Nickerson (Canada).

The validity of conclusions regarding the relation of chemical structure to biological activity appears to relate directly to the precision with which observations can be related to a specific action on the biological system. Two distinct steps must be considered in any such action: association with a specific "receptor" (affinity, 1/K) and alteration of the biological system in such a way as to produce a measurable effect (intrinsic activity), both of which may vary continuously over a wide range. Factors responsible for differences in intrinsic activity may be more important for understanding mechanisms of drug action than are the apparently less rigorous requirements for simple attachment to receptor sites. A minimum requirement is that these two factors be distinguished and evaluated separately.

Interpretations also must recognize the diversity of structural requirements for activation of blockade of receptors subserving different responses to a single general type of pharmacological agent. General designations such as adrenergic, cholinergic or histamine receptors appear to be inadequate. Differences within each of these groups may be as great as those between them, e.g. muscarinic vs. nicotinic cholinergic receptors, α vs. β adrenergic receptors, histamine receptors mediating smooth muscle contracting vs. those mediating gastric secretion. It is of interest that antagonists may distinguish sharply between receptors activated by the same or very similar agonists. Any fully valid theory relating chemical structure to biological activity must encompass and provide explanations for differences in affinity and intrinsic activity and in the structural requirements for activation or inhibition of similar receptors.

102 Nature of the Cholinergic Receptor. P. G. Waser (Switzerland).

In order to investigate the existence, distribution and nature of cholinergic receptors different drugs were labelled with ^{14}C and their concentration and distribution in end-plates of mouse diaphragms demonstrated by autoradiography. Several million molecules of calabash curarine, toxiferine and decamethonium are combined with each end-plate and the number of molecules bound is related to nerve–muscle blockade. The cholinergic receptors are saturated with curarine when nerve–muscle blockade is complete. Only a few highly differentiated and widely distributed receptors are occupied on the postsynaptic membrane. Denervation experiments showed a relationship between curarine binding and cholinesterase. For depolarization more molecules than for blocking action are needed. Antagonism between neostigmine, acetylcholine, potassium and curarizing drugs on the receptor has been investigated.

103a Discussion of Previous Paper. S. Ehrenpreis (U.S.A.), C. Chagas (Brazil) and T. M. Turpajev (U.S.S.R.).

103b Protein Nature of the Acetylcholine Receptor and the Role of Macroergs in the Realization of Acetylcholine Action. T. M. Turpajev, T. G. Putintseva and S. N. Nistratova (U.S.S.R.).

On the intact *Rana temporaria* ventricle, data were obtained proving protein nature of the acetylcholine receptor, the substrate of the primary reaction between the mediator and effector cell. At 40°C, heat denaturation of the receptor takes place. Its irreversible stage is not accompanied by cholinesterase inactivation, which contradicts the ideas on the identity of cholinesterase and acetylcholine receptor. Upon cooling to zero, the receptor undergoes a change into an inactive state; this inactivation being accompanied by a reversible change in the receptor structure. Two types of reaction occur between acetylcholine (A) and the receptor (R): A R AR and 2A R A_2R. The receptor contains free SH-groups the reactivity of which decreases upon interaction with acetylcholine. By means of this biochemical test the receptor was found and isolated from the water-soluble protein fraction of tissue homogenate.

The biochemical nature of the receptor protein representing a substrate for the action of many pharmacological substances has been studied.

Like acetylcholine, atropine decreases the reactivity of the SH-groups of the receptor molecule active centre. Affinity of the receptor to atropine is about 100 times that to acetylcholine.

When acetylcholine acts on the intact ventricle of the frog heart and on the isolated rabbit auricle, a stimulator of heart activity of non-sympathetic nature is released. Uncoupling drugs, 2:4-dinitrophenol and sodium azide inhibit the liberation of the stimulator. The action of this stimulator upon the heart is similar to that of uridin-di- and triphosphate.

The data obtained are discussed in the light of Koshtoyants' enzymo-chemical hypothesis.

104 Correlation between Metabolism and Behavioural Action of Psychotropic Tryptamine Derivatives. S. Szára (U.S.A.).

N.N-dimethyltryptamine and its N.N-diethyl and N.N-dipropyl homologues produce autonomic symptoms, perceptual, emotional, and thinking disturbances in man (in doses of 1 mg/kg) similar to LSD_{25} or mescalin but for a much shorter period of time. The corresponding dibutyl derivative causes only very slight symptoms while the dihexyl compound is completely inactive in the same dose.

The psychotropic activity of these homologue derivatives was found to be dependent primarily upon the rate of their metabolism in the liver.

Besides MAO, for which these tertiary amines are poor substrates, they are attacked by two competing microsomal enzymes both requiring TPNH, and O_2.—6-hydroxylation produces psychotropically active metabolites, while dealkylation forms practically inactive compounds. 6-hydroxylation is inhibited, among others, by chlorpromazine, reserpine, and by the substrate itself at higher concentrations, while the dealkylation reaction follows the Michaelis-Menten law. The inhibition of 6-hydroxylation by substrate is more pronounced in the case of the higher homologues; the dibutyl derivative is hydroxylated very slowly and the dihexyl compound is not hydroxylated at all.

Thus a definite correlation exists between the ability of a derivative to serve as substrate for the 6-hydroxylation reaction and its behavioural and psychotropic activity. This points to the significance of the formation of a 6-hydroxylated metabolite in producing behavioural and psychological symptoms for which evidence from animal and human experiments will be presented.

The question will also be discussed how new drugs with direct and indirect psychotropic activity could be designed from metabolic considerations.

105 5-Hydroxytryptamine and 5-Hydroxytryptophan Decarboxylase Activity in the Developing Nervous System of Rats and Guinea-Pigs. S. E. Smith, R. S. Stacey and I. M. Young (United Kingdom).

The concentration of 5-hydroxytryptamine (5-HT) and 5-hydroxytryptophan decarboxylase activity has been examined in foetal, neonatal and adult rats and guinea-pigs. Only whole brains were used. 5-HT was assayed fluorimetrically and decarboxylase was estimated by the formation of 5-HT on incubation of whole homogenates at pH 8 with excess 5-hydroxytryptophan and pyridoxal-5-phosphate.

In both species the amount of decarboxylase activity at birth approached the adult figure. This was also true of the amount of 5-HT stored in the brains of guinea-pigs at the same stage of development. Even during the latter half of foetal life the 5-HT concentration was as much as half that of the adult and rose during the first few weeks of extra-uterine life. This finding contrasts strikingly with the very low concentrations of 5-HT found by Nachmias in the neonatal rat.[1]

In the brain there appears to be a dissociation of decarboxylase activity (which, unlike that in the kidney,[2] develops very early in foetal life) from 5-HT storage which increases much later. At birth, guinea-pigs are relatively well developed whereas rats are very immature. The results suggest a correlation between the amount of 5-HT in the brain and the degree of maturity.

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106 Effect of Reserpine and the Metabolism of Serotonin in Tryptophan Deficient Rats. E. M. Gal, P. A. Drewes and C. A. Barraclough (U.S.A.).

Rats kept on tryptophan-free diet for 6 weeks showed about 70 per cent lower serotonin in the brain than their tryptophan fed controls. A single injection of 5 mg/kg of reserpine phosphate produced sedation and 80 per cent mortality in these chronically deficient rats. Mitochondrial monoamine oxidase activity was only increased in the liver after 4 weeks on deficient diet. When rats were given on the ninth day of dieting a single dose of 5 mg/kg of reserpine, the controls regained their normal serotonin and norepinephrine levels by the twenty-first day while those on the deficient diet remained serotonin depleted. By this method, serotonin-depleted rats could be produced well before the onset of chronic tryptophan deficiency with less than 5 per cent mortality. The serotonin levels were 75 per cent lower in the brain and the spleen, 70 per cent lower in the blood and 60 per cent lower in the small intestine than those of the controls. There was no meaningful difference in the norepinephrine content of the brain between the serotonin depleted and the control animals. A second injection of 1-2 mg/kg of reserpine on the twenty-third day produced sedation in both groups of rats. EEG studies failed to reveal any difference in the hypothalamic activity or in thresholds of arousal and recruiting in either control or in the depleted rats after administration of reserpine. It is concluded that the control nervous system action of reserpine does not require the presence of serotonin in rat brain.

107 Comparative Effects of α-Ethyltryptamine Isomers on Certain Systems in vitro and in vivo. M. E. Greig, A. J. Gibbons, J. P. DaVanzo and O. K. Sebek (U.S.A.).

The D- and L-isomers of α-ethyltryptamine (α-ET) are monamine oxidase inhibitors of the competitive type. When tested *in vitro*, both forms were equally effective in suppressing the oxidation of

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serotonin (5-hydroxytryptamine). Acute experiments *in vivo* in rats showed that D,L-α-ET alone caused an approximately 30 per cent increase of the brain serotonin in 1 hr. When the administration of D,L-α-ET was followed by 5-hydroxytryptophan (5-HTP) much higher levels of the brain serotonin were found. These were dependent on the doses of both α-ET and 5-HTP. When D,L-α-ET was followed by tryptophan (TP) the levels of brain serotonin also increased, but in this case higher doses of the inhibitor tended either to lower or to cause no further increase of brain serotonin. Since α-ET caused a maximum level of serotonin accumulation only when administered with 5-HTP, but not with TP, it was postulated that larger doses of α-ET inhibited 5-hydroxylation of TP. Support for this hypothesis was provided by determining the effect of α-ET on the synthesis of violacein, a bacterial (*Chromobacterium violaceum*) pigment, the development of which is dependent on 5-hydroxylation of TP. The amount of pigment formed was inversely proportional to the amount of either α-ET isomer, the D-form being more inhibitory. In chronic experiments with rats D,L-α-ET caused increases of brain norepinephrine. Polydipsia of unknown origin was caused only by the L-isomer. In dogs the D-form caused a greater stimulation of the central nervous system than the L-isomer. Additional testing is necessary before the significance of these observation can be determined.

108 Interactions of Serotonin and Other Indoles with Pyridine Coenzymes and Related Structures. S. G. A. Alivisatos (U.S.A.).

Diphosphopyridine nucleotide (Coenzyme I) and other "onium" bearing structures interact non-enzymically with various indoles including serotonin and tryptophan (charge-transfer complexes). When these phenomena were first observed in our laboratory,[1] they became apparent by the visual appearance of a yellow-greenish coloration. The spectroscopic counterpart of the visual impression was a broad, featureless absorption band extending from beyond 400 mμ to at least 300 mμ (transfer-spectrum).[2] The high absorption of both DPN and the indoles at the concentrations required for *in vitro* interactions prevented extension of the curves below 300 mμ. Kinetic and equilibria considerations[3] led to the conclusion that at least in the pH range 2·0 to 8·0 these interactions occur between one coenzyme molecule and one molecule of the indole. It was also shown that in this pH range there is not much difference in the intensity of the colour developed upon admixture of the reactants. However, at pH values above 8·0 the colour intensity increased rapidly. The equilibrium constants of these associations have been determined. Also, the dependence of the associations on various other groupings present in the interacting species, like the ethylamine residue of serotonin and the 6-aminopurine of coenzyme I, have been studied. The coenzyme I–tryptamine complex was isolated in relatively pure form and its properties were studied. The mechanism of these phenomena and their possible relation to *in vivo* interactions will be discussed.

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2. *Nature* (*Lond.*), **186,** 718 (1960); **187,** 374 (1960).
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109 The Influence of Some Indole Derivatives on the Activity of Choline Acetylase and CoA. B. Bošković and R. Pržić (Yugoslavia).

We have investigated the influence of the following substances on the activity of choline acetylase and CoA: *o*-phosphoryl-4-hydroxy-N-dimethyl tryptamine (Psilocybin); 5-hydroxytryptamine (5-HT); D-lysergic acid diethylamide (LSD_{25}); 2 - methyl - 3 - ethyl - 5 - dimethylaminoindole (Medmain); 2-brom-lysergic acid diethylamide (BOL); 7-methoxy-1-methyl-9 pyrid (3:4-β)-indole and adrenochrome.

We have found that Psilocybin, harmine and LSD_{25} in the concentration of 1×10^{-7} M do not have any effect on the activity of choline acetylase. 1×10^{-6} M in the system potentiates the action of the enzyme. BOL, 5-HT and Medmain are without effect on the activity of choline acetylase in the conc. of 1×10^{-7} M, 5×10^{-7} M and 1×10^{-6} M adenochrome (5×10^{-7} M) shows some inhibitory effect.

Psilocybin, LSD_{25} and Medmain in the concentrations of 1×10^{-7} and 1×10^{-6} M in the system have no effect on the activity of CoA. 5-HT in the concentrations of 1×10^{-7} M, 5×10^{-7} M and 1×10^{-6} M inhibits CoA, the percentage of inhibition depending on the concentrations used. BOL, 1×10^{-7} M, has no effect on the activity of CoA, but at the conc. of 1×10^{-6} M shows some inhibitory effect. Adrenochrome, 1×10^{-6} M, also shows some inhibitory to determine by the method used because of chemical interference by harmine with the other reagents.

110 Reserpine and Cardio-Vascular Centres. C. Heymans, A. F. de Schaepdryver, G. R. de Vleeschhouwer, A. C. Taquini, jr., and J. B. van der Schoot (Belgium).

Pharmacological effects of reserpine on cardiovascular centres have been studied in 35 mongrel dogs, anaesthetized with morphine-chloralosane.

Intracisternal injections of reserpine (0·1 mg/kg) induce, in most cases, a moderate increase of the general arterial blood pressure and a marked sensitization of the carotid sinus vasomotor reflexes.

Injections of reserpine (20 mg) into the arterial circulation of the isolated perfused head, connected with the body by the vagi only, provoke a pronounced sensitization of the cardio-inhibitory centre to both reflex and direct stimulations.

Injections of reserpine (20 mg) into the arterial

circulation of the isolated perfused head, connected with the body by the spinal cord only, are followed by an immediate and long-lasting stimulation of the vasomotor centres, and by a pronounced and progresively increasing potentiation of the carotid sinus vasomotor reflexes.

Biochemical data, including estimations of catecholamines and serotonin in the central nervous system and of the peripheral catecholamines, will be reported and correlated with the observed effects of reserpine on the cardio-vascular centres.

111 Amino-Acid and Carbohydrate Metabolism of Brain after Reserpine. D. Palm, H. Balzer and P. Holtz (Germany).

In white mice reserpine caused significant changes in the GABA-content of brain. There was good correlation between the development and the duration of the decreased GABA-content of brain and the lowered threshold for electro-shock. Iproniazid prevented both actions of reserpine: it normalized the GABA-content and the threshold for electro-shock.

Whether there exists a relationship between these results and changes in the amine and carbohydrate metabolism of the brain caused by reserpine will be discussed.

112a Un Nouvel Antagoniste de la 5-Hydroxytryptamine (5-HT). R. Cahen, J. Nadaud et S. Aubron (France).

Les antisérotonines sont, soit des homologues synthétiques de 5-HT, soit des alcaloïdes naturels. Nos observations sur l'effet dépresseur sur le muscle lisse du nébulisat d'aubier de tilleul (T) nous ont conduit, et afin de répondre aux questions posées par les observations de certains cliniciens, à étudier l'effet antisérotonine en comparaison de LSD_{25}, B.A.S., T.D.A.

(1) Organes isolés (étudiés selon la technique de Gaddum): De même que LSD_{25} et B.A.S., T inhibe l'effet de 5-HT et non de l'adrénaline sur l'oreille isolée de lapin; de 5-HT et non de l'ocytocine sur l'utérus de rate; de 5-HT et non de l'acétylcholine sur l'iléon de cobaye. Utilisant la méthode de Gaddum *et al.*, on observe que l'effect s'exerce principalement sur les récepteurs tryptaminiques musculaires (D).

(2) Action vasculaire et intestinale: Chez le chien chloralosé, comme LSD_{25}, T inhibe l'effet hypertenseur et spasmogénique intestinal de 5-HT. Chez la souris, comme B.A.S., T inhibe l'effet laxatif de 5-HT.

(3) Action centrale: Contrairement aux doses élevées de LSD_{25} et de même que T.D.A. et B.A.S., T n'inhibe pas l'effet potentialisateur de 5-HT sur l'analgésie morphinique, le sommeil barbiturique chez la souris et l'hyperthermie par la sérotonine endogène (5 HTP + INH) chez le lapin. Au contraire et comme les doses minimes de LSD_{25} il exerce une potentialisation. On peut supposer que, comme T.D.A. et B.A.S., T ne franchit pas à concentration suffisante, la barrière hématoencéphalique.

Conclusions. T est antagoniste des effets périphériques et non centraux de 5-HT; il est comparable à T.D.A. et B.A.S.

112b A New Antagonist of 5-Hydroxytryptamine (HT). R. Cahen, J. Nadaud et S. Aubron (France).

Antagonists of HT are either synthetic analogues of HT or natural alkaloids. Our findings that an aqueous extract of lime-tree laburnum has a depressant action on smooth muscle prompted the investigation of an antagonism of HT, as compared with LSD_{25}, B.A.S. and T.D.A.

(1) Isolated organs: Using Gaddum methods, the same as LSD_{25} and B.A.S., T antagonizes the constrictor effect of HT but not of epinephine on perfused rabbit ear; of HT induced contraction but not of ocytocin on rat uterus; of HT but not of acetylcholine on guinea-pig ileum. Experiments by the Gaddum *et al.* methods lead to the conclusion that T acts chiefly on the muscular tryptamine receptors (D).

(2) Pressor and intestinal effects: Just as LSD_{25}, T antagonizes HT effects on blood pressure and gut *in situ* in dogs under chloralose. In mice, as with B.A.S., T antagonizes HT effect on faecal excretion.

(3) Central effects: Contrary to high doses of LSD_{25}, but like T.D.A. and B.A.S., T does not antagonize the potentiating effect of HT on morphine analgesia, on hexobarbital hypnosis in mice, on pyretogenic action of endogenous serotonin (HTP + INH) in the rabbit. Just as small doses of LSD_{25}, T enhances such effects. It may be supposed that like T.D.A. and B.A.S., T does not penetrate into central nervous system tissue in sufficient quantity.

Conclusion. T antagonizes specifically the peripheral effects of HT but not the central ones.

113 Reserpine: Modification of its Tranquilizer Effect and Analysis of its Central Mode of Action. J. Knoll and B. Knoll (Hungary).

It has been shown that the deconditioning effect of 1 mg/kg of reserpine in rats maintained at 4°C did not differ substantially from that seen in rats kept at room temperature. Similarly, the inhibition of the morphine-induced hypermotility by reserpine (1 mg/kg) remained unchanged in animals exposed to cold stress. It has also been shown that in most rats pretreatment with iproniazid did not inhibit the deconditioning effect of 10 mg/kg of tetrabenazine or 2 mg/kg of reserpine. It only caused a characteristic delay in the development of the effect. A similar action is exerted by cocaine (20 mg/kg) and phenmetrazine, respectively.

The deconditioning activity of reserpine has been determined quantitatively by the jumping test[1] and the inhibition of hypermotility by the drug was

measured quantitatively by means of the motimeter.[2]

From the data in the literature and the evidence obtained from the present investigation, it was concluded that the action of reserpine has two components, opposite in direction. One is a central stimulant action of short duration and the other a depressant action, which lasts for an extremely long time. It would appear that the central stimulant action is in causal relationship with the liberation of biogenic amines, i.e. it is an indirect effect, whereas the central depressant (tranquilizer) effect is the action of the molecule itself (a *per se* effect).

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114 On the Role of Serotonin and Catecholamines in Relation to Some Effects of Reserpine. J. NEČINA and I. KREJČÍ (Czechoslovakia).

It is generally known that the formation of gastric ulcers after reserpine can be inhibited by pretreatment with iproniazid. As a mono-amine-oxidase inhibitor we used in our study, methylene blue as well as iproniazid and the results were identical.

To differentiate the role or serotonin and catecholamines, in further experiments we selectively increased their tissue levels.

According to Brodie and Garattini, animals when kept at a temperature of 0–4 °C showed after reserpine neither serotonin depletion nor sedation, whereas its depletion of catecholamines usually followed. In our experiment, rats after being injected with reserpine were exposed to cold stress for 6 hr. The number of ulcers was significantly higher than in the controls. We concluded that lowering of sertonin is not responsible for the ulcer formation. On the contrary, the production of ulcertations was prevented completely by increasing catecholamine levels with DOPA.

Apart from this we found that the number of white mice which died in the temperature of 0 °C during 5 hr after the application of reserpine was significantly reduced after methylene blue. The effect of DOPA was similar. The role of serotonin in this process can be ignored for there is no depletion of serotonin in the cold.

From the results of our experiments we draw attention to the important role of low catecholamine levels in the formation of gastric ulcers after reserpine and its higher toxicity in low temperature.

115 On the Mode of Action of Cardiac Glycosides. A. I. CHERKES (U.S.S.R.).

Accumulated data on metabolism of the heart muscle and the discovery of the role of its disturbances in the development of cardiac pathology induced the pharmacologists to study the mode of action of cardiac glycosides with special reference to their effect on the metabolism of the cardiac muscle.

In this laboratory the effects of cardiac glycosides on myocardial respiration, oxidation substrate utilization, carbohydrate and phosphate metabolism and activity of several myocardial enzymes under normal and pathological conditions (investigations *in vivo*, acute and chronic experiments *in vivo*) on different animal species have been studied during a number of years.

Cardiac glycosides increase oxygen uptake and utilization of oxydation substrates by myocardial tissue. The administration of cardiac glycosides results in the increase of respiratory activity of the diseased heart tissue (myocarditis, cardiac failure) to the normal level with simultaneous normalization of the ECG.

Cardiac glycosides in therapeutic doses produce an increase in glycolysis, inhibition of phosphorylase activity and enhancement of myocardial lactate utilization for glycogen resynthesis.

In the contracting heart (heart-lung preparation, the heart *in situ* with a catheter introduced into the coronary sinus) myocardial lactic acid uptake from the inflowing blood increases under the influence of cardiac glycosides and this process, disturbed in the failing heart, returns to normal concomitant with the disappearance of cardiac failure.

Cardiac glycosides exert only a slight influence on the energy-rich phosphate content possibly owing to the fact that both breakdown and resynthesis of adenosine triphosphate are activated by these compounds.

Our understanding of the intimate nature of cardiotonic action of glycosides depends upon the development of our knowledge of intermediary metabolism of the heart muscle on a molecular level and of physical and chemical phenomena underlying the normal cardiac activity and its disturbances. It is only the deep inquiry into all these questions that will pave the way for rational use of glucosides in cardiology and for its enrichment with new methods and means.

116 Differential Action of Dihydro-Ouabain and Ouabain. M. TAESCHLER and A. CERLETTI (Switzerland).

DH-Ouabain, when administered by constant infusion, in the dog heart-lung preparation produces its positive inotropic effect with a smaller proportion of its lethal dose than does ouabain.[1] Experiments on the isolated, spontaneously beating guinea-pig auricle as well as on the heart-lung preparation were performed in order to analyse further the nature of this greater margin between therapeutic and toxic dose of DH-ouabain.

The two glycosides were slowly infused into the Ringer solution containing the isolated, spontaneously beating auricle. The ratio of the minimal effective concentration (eliciting a positive inotropic effect) to the toxic concentration (eliciting arrhythmia and contracture) averaged 1:5·3 for ouabain

and 1:14 for DH-ouabain, showing a greater safety margin for DH-ouabain.

In subsequent experiments on the isolated auricle, single doses of the two glycosides were administered. As the concentration of ouabain decreased, the delay in the onset of both inotropic and toxic effects was increased. The cardiac effect is thus determined by the product of concentration x duration of exposure to the glycoside. By contrast, the cardiac effects of DH-ouabain, if present, were always immediate and largely independent of the exposure time. Threshold concentrations therefore exert inotropic effects, but not toxic effects, whatever the duration of exposure. In contrast to ouabain, DH-ouabain does not exhibit cumulation.

Similar results were observed in the heart-lung preparation.

This difference in action between the natural and the hydrogenated glycoside explains, at least partly, the increased therapeutic: toxic ratio determined by an infusion method.

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117 Influence of Cardiac Poisons and of some of their Inactive Derivatives on Membrane ATPase. H. J. Portius and K. Repke (Germany).

Recently, we have found that the well-known differences in biological activities of digitoxin and dihydrodigitoxin are not related to differences in their metabolic fate but must be due to differences in their capacities to modify "receptor sides". Trying to characterize the biochemical nature of "receptors" we studied the influence of cardiac poisons and that of some of their inactive derivatives (digitoxin and dihydrodigitoxin; digitoxigenin, 3-dehydrodigitoxigenin, 3-epidigitoxigenin and *iso*-digitoxigenin; cymarin and 17α-cymarin; strophanthidin and *iso*strophanthidin) on membrane ATPase of human erythrocytes activated by Mg^{++}, Na^{+} and K^{+}. The experimental conditions for measuring ATPase activity were the same as those described by Post *et al.*,[1] but the Mg^{++}/ATP ratio was 2:1.

As far as there was any effect, only inhibition has been observed. The concentrations needed for half-maximal inhibition varied with the different compounds over a wide range but were mostly parallel to biological activities. Ca^{++} effected an inhibition of the ATPase also. The cardiac poisons strengthened its inhibitory effects.

The results suggest the idea that the differences in biological activities between the cardiac poisons and some of their derivatives are possibly due to their different capacities to influence enzymatic systems.

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118 Improved Intestinal Absorption of Polar Cardiac Glycosides by Acetylation. R. Megges and K. Repke (Germany).

Present evidence favours the idea that the velocities of intestinal absorption will increase with decreasing polarities of glycosides.[1] To prove this idea the absorption of moderately polar glycosides as compared with some of their acetylated derivatives were studied in the rat. Rising degrees of acetylation increasingly reduced polarities of molecules. This was reflected by increasing mobilities in paper chromatographical separation with formamide systems.

The compounds dissolved in a small volume of pyridine were injected intraduodenally with a micrometer syringe. Simultaneously, a suspension of rice flour coloured with Indian ink to indicate the speeds of peristaltic transport, was injected into the stomach. At different intervals, the amounts of the original compounds and metabolites in tissues and excreta were determined by microchemical methods.

As a rule, the degree of intestinal absorption has been found to increase with rising degrees of acetylation. The acetylated derivatives will be excreted in minor amounts in bile or urine. Acetylation varying in degree or nature thus favour maintenance of an active blood level in an almost predictable manner. Enzymatic deacetylation already beginning in the alimentary canal will be chiefly accomplished by liver. If not further transformed, the free glycosides will now be excreted in bile or urine. Some glycosides as gitoxin which are ineffective when used orally can be absorbed by transformation into their peracetylated derivatives.

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119 Digitalis–Ryanodine Antagonism. S. Hajdu and E. Leonard (U.S.A.).

The depressant effect of ryanodine on myocardial contractility of strips of rat right ventricle was examined. Contractility of fresh rat heart varies with stimulation frequency. It is greater at high and low rates of stimulation and lesser at intermediate rates. The high contractility observed at low stimulation rates is eliminated by prolonged washing. Normal contractility of washed hearts is restored either by cardiac glycosides or by K-free solutions. Within 15–20 min of addition of ryanodine to fresh rat heart, force–frequency relations characteristic of the long-washed heart appear. The action of glycosides on contractility is rapidly reversed by ryanodine, and additional glycoside does not restore contractility. The ryanodine effect can be counteracted, however, by removal of K from the bath. Large myocardial twitch tension produced by rapid stimulation is associated with a net loss of K, and the drop in tension on returning to slow stimulation is accompanied by cellular K gain. It is thought that digitalis maintains high

tension at low rates of stimulation by slowing the rate of K re-entry, and that ryanodine, with its opposing effect, hastens re-entry. Ryanodine action can thus be inhibited if extracellular K is low. Digitalis–ryanodine antagonism led to studies which show that in anaesthetized cats, otherwise fatal ventricular tachycardia induced by intravenous digitoxin can be abolished by ryanodine. However, following suppression of ventricular arrythmia, the cardiac cycle is initiated by a supraventricular focus, frequently nodal.

120 Effects of Cardiac Glycosides on the Hyperpolarization which follows Activity in Nerve Fibres. H. W. Böhm and R. W. Straub (Germany).

In nerve fibres of small calibre, a burst of activity gives rise to a long-lasting period of hyperpolarization which appears to be caused by the increased extrusion of sodium ions which follows activity.[1, 2] Previous studies in mammalian non-myelinated (C) fibres have shown that the post-tetanic hyperpolarization is abolished by ouabain within a few min, before the resting membrane potential is appreciably decreased.[1] We have now found that the post-tetanic hyperpolarization in amphibian myelinated fibres is also reduced by cardiac glycosides, before the resting or the action potential is decreased, but the poisoning develops much more slowly. A small decrease only was usually seen when myelinated fibres were poisoned for a few min with ouabain or with digoxin (5μM), and even when the preparations were stimulated continuously it took 1–2 hr until the hyperpolarization was abolished. A slowly progressive decrease of the hyperpolarization was also found when myelinated fibres were exposed to K-free solution; the inhibition by K lack developed much more slowly than the glycoside poisoning. The slowness with which K deprivation or cardiac glycosides act in myelinated fibres—in contrast to the rapidity with which they inhibit K uptake in many tissues,[3] and the post-tetanic hyperpolarization in C fibres—suggests that the ion transport mechanism responsible for the hyperpolarization in myelinated fibres is not easily accessible to the glycosides, possibly because active transport in the myelin-covered parts of the axons contributes to the hyperpolarization. Another possibility would be that the hyperpolarization is predominantly due to uncoupled (electrogenic) Na extrusion,[2] which perhaps is not affected by cardiac glycosides.

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121 The Influence of Therapeutic and Toxic Concentrations of Digitoxigenin on the K-Flux and the Ion Concentrations in Isolated Guinea-Pig Atria. W. Klaus, G. Kuschinsky and H. Lullmann (Germany).

One of the common conceptions about the mode of action of digitalis glycosides is that these substances primarily influence the K-exchange across the membrane of the heart muscle cell, therewith producing the conditions which engender a positive inotropic effect.

This theory was checked under defined conditions by means of ^{42}K with isolated guinea-pig atria (Tyrode solution, 30°C, constant stimulating frequency). Determinations have been made of the K-efflux under simultaneous registration of the mechanogram, of the K-influx, and of the K-, Na-, Cl-, and Ca-concentrations with control and digitoxigenin (10^{-7}–10^{-6} g/ml) treated atria.

The K-efflux of untreated atria has been found to be 0·9 %/min (mean), and was—as the K-influx—not influenced by therapeutic concentrations of digitoxigenin (with positive inotropic action only). Accordingly, the ion concentrations after a 60 min stay in the respective solutions was not significantly altered: controls, K = 65 ± 2 ($\bar{x} \pm s_{\bar{x}}$), Na = 74 ± 2; digitoxigenin 3×10^{-7}, K = 63 ± 2, Na = 66 ± 2 meq/kg wet wt. Contrarily, toxic concentrations of digitoxigenin (permanent contracture) produced an increase of the K-efflux, e.g. with digitoxigenin 10^{-6} by the factor 1·5–2. The K-influx is slightly decreased under these conditions. Correspondingly, the atria showed a decrease in K-concentration and a roughly equivalent increase in the Na-concentration (49 ± 3 and 82 ± 3 meq/kg wet wt. respectively). The Cl- and Ca-concentrations were not significantly altered.

It is concluded that the influence of digitoxigenin on the K-fluxes and the concentrations of the ions determined cannot be the cause of their positive inotropic effect but merely constitute non-specific toxic phenomena.

122 The Influence of Dihydro-Ouabain on the Contraction of the Heart Muscle in Dependence on Temperature and Calcium-Concentration. M. Reiter (Germany).

Measurements of contractions of electrically stimulated (1/sec) papillary muscles of guinea pigs have shown that the influence of dihydro-ouabain (DHO) in successive concentrations (5×10^{-8}; 1, 2, 4 and 8×10^{-8} M/l) on the velocity of shortening and on peak-tension depends on the calcium concentration, i.e. whether Ca causes already a maximum of velocity of shortening or not. This maximum is reached at 18° and 25°C with lower Ca-concentrations than at 35°C. Therefore, at 35°C DOH causes at 1·2 and 2·4 mM/l. Ca a strong increase of velocity and of amplitude of contraction despite a shortening of contraction time, while at 25°C at the same Ca-concentrations tension may

even decrease due to the shortening of the contraction time, and only at 0·6 mM/l. Ca DHO causes a dose-depending increase of contraction force. Similar relations prevail at 18° between 1·2 and 2·4 mM/l. Ca for the first three DHO-concentrations. With the dose-depending shortening of the contraction time at higher DHO-concentrations (4 and 8×10^{-5}, at 35° occasionally at 2×10^{-5}) after-contractions appear, the amplitudes of which are at 35°C almost as high as the original one, and lower at 25°C, but more closely connected to the relaxation phase of the previous contraction. At 18° the after-contraction seems to be merged with the original contraction: the time of the whole contraction increases strikingly, leading to an increase of tension.

From the action of the glycoside on the different parameters of contraction, only the shortening of the contraction time seems to be related to the concentration of the glycoside (with the exception of the highest concentrations at 18°) regardless of the Ca-concentration, which may at a given temperature be too high as to allow an increase in tension.

123 Increase in Sodium Excretion from the Unilateral Kidney Produced by Ouabain Injected into the Renal Artery in Dogs. T. Tanabe, I. Tsunemi, Y. Abiko and S. Iida (Japan).

In dogs hydrated with saline, an injection of ouabain into the unilaterial renal artery produced a continuing marked increase in excretions of water, sodium and chloride from the kidney, but not from the counter kidney. The resulting unilateral diuresis was not due to changes in systemic blood pressure, and it was confirmed, by direct measurement of blood flow and with clearance technics, that ouabain produced a decrease in the renal blood flow by vasoconstriction and in GFR. Ouabain did not change Tm_G and exretion of ammonia, nor did it significantly enhance potassium excretion in dogs as it does in rats. Further, the action of ouabain was not influenced by preliminary administration of anti-aldosterone steroids. Ouabain did not suppress the effect of desoxycorticosterone glucoside injected into the same renal artery.

After dogs were hydrated with isotonic sucrose solution, ouabain failed to produce a marked increase in urine volume as seen in dogs hydrated with saline. However, since sodium concentration was markedly increased in urine samples obtained from the kidney of the injected side, we can say ouabain induced a pronounced natruresis unilaterally. Chloride also increased, but potassium did not.

It is suggested that ouabain has a direct inhibitory effect on the reabsorptive mechanism of sodium in tubules and the effect may not be explained by antagonistic action against aldosterone.

124 Effect of Cardiac Glycosides on the Active Biological Transport of Non-electrolytes. T. Z. Csáky (U.S.A.).

L-Tyrosine, 3-0-$^{14}CH_3$-glucose, DL-phenylalanine-2-^{14}C and uracil -2-^{14}C are transported against higher concentrations into the serosal compartment in the surviving isolated intestine of the frog. Ouabain (10^{-4} M) markedly inhibits the transport of all 4 non-electrolytes. This action parallels the inhibition caused by the replacement of Na ions with Li or K ions in the bathing medium. Thevetin has the same qualitative and quantitative effect as ouabain, whereas hexahydroscillaren A inhibits the transport only in concentrations of 10^{-3} M or higher. The small intestine of anaesthetized guinea pigs was perfused *in situ* with iso-osmotic Na_2SO_4 solution containing 50 mg per cent glucose. In this preparation, rapid active glucose transport takes place from the lumen into blood that has a glucose content of 100 mg per cent or more. The rate of this transport is reduced following an intravenous injection of 0·15 mg/kg ouabain or by adding digitoxin in 10^{-4} M to the perfusing fluid. However, in similar preparations the absorption of substances which are not likely to be actively transported such as ethanol or antipyrine is also inibited by ouabain or digitoxin. The results are discussed in view of: (a) the known dependence of the active intestinal transport of non-electrolytes upon Na ions[1, 2, 3]; and (b) the possibility of a direct inhibition of all active transport processes by cardiac glycosides.

1. Csáky and Thale (1960), *J. Physiol.*, **151,** 59.
2. Csáky and Zollicoffer (1960), *Amer. J. Physiol.*, **198,** 1056.
3. Csáky (1961), *Fed. Proc.*, **20**, 139.

125 Effect of Rapid Acting Heart Glycosides on Electrolyte Balance of Myocardium. N-H. Areskog (Sweden).

Studies were performed on the dog heart-lung preparation. Plasma Na, K and Ca were determined by flame photometry. In some experiments radioactive ^{24}Na and ^{42}K were used. After an equilibrium period of 60–90 min, the heart glycoside (acetyl strophanthidin or convallatoxin) was injected. The radioactivity of plasma, blood and myocardial samples was determined by gamma-spectrometry. The pH was varied by altering the P_{CO_2} of the inspired air. Toxic doses of the glycosides were used.

A great loss of K from the myocardium was induced by the glycosides but there was not a corresponding loss of ^{42}K. This result would be explained if a two-compartment system for the myocardial K exists, one small and easily exchangeable, and one larger and more firmly bound fraction which is liberated by the glycosides. The loss of K from the myocardium seemed to be inversely proportional to pH.

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In further experiments isolated atrial strips or papillary muscles from rabbit hearts were first loaded with ^{42}K. Some of the strips were then exposed to a heart glycoside in Ringer solution, while others served as controls. The loss of ^{42}K was determined.

In the heart-lung preparations, the total plasma Na was unchanged, or showed a slight increase with the glycosides. This indicates that only small changes of the myocardial Na balance took place. This might at least partially be explained by an increase of the hydrogen ion production in the myocardial cells due to the glycosides. The ^{24}Na movements were generally in antiphase to ^{42}K.

The Ca content of plasma was not changed by the glycosides.

126a Contribution à l'Etude de la Maturation Cérébrale chez l'Animal. Effet des Drogues Neurotropes. C. Radouco-Thomas, F. Martin, S. Radouco-Thomas, G. Nosal et J. M. Chaumontet (Suisse).

Dans des recherches antérieures, nous avons étudié chez le rat les relations entre le développement post-natal de l'activité bioélectrique cérébrale, le comportement et la capacité conditionnante.[1] Dans de travail, nous examinons les rapports existants entre l'électro-ontogénèse et certains aspects de la maturation morphologique et biochimique du cortex cérébral du rat depuis la naissance jusqu'à l'âge adulte.

Les recherches sont effectuées sur des séries longitudinales et horizontales de rats W.A.G. à des âges déterminés de 0, 4, 7, 10, 12, 14, 24, 30, 90 jours. L'activité bioélectrique cérébrale est définie par l'EEG spontané et le potentiel strychninique. L'ontogénèse de l'EEG spontané semble présenter une série de paliers maturatifs vers les 4e, 7e et 14e jours. Sur le plan morphologique (préparations Nissl, Weigert, Golgi-Cox), l'étude de l'ontogénèse de la neuropile corticale est effectuée sur le triple aspect: périkarionique, dendritique et axonique. L'evaluation quantitative de la maturation morphologique du néocortex est en cours d'étude.

Dans le cadre des recherches sur le métabolisme cérébral, la consommation d'O_2 par le cerveau de jeune rat est examinée dans la période post-natale, en corrélation avec les moments critiques de la maturation électro-ontogénique et structurale. Des lésions corticales expérimentales (décortication chimique) sont provoquées à différents âges chronologiques et leur effet est étudié en relation avec l'électro-ontogénèse et la capacité conditionnante. L'action des neurostimulants et neurodépresseurs, ainsi que l'effet de l'ACTH, est examinée sur l'EEG spontané et la consommation d'O_2 pendant le développement post-natal du cerveau de rat.

126b Contribution to the Study of Cerebral Maturation in Animals. Effect of Neurotropic Drugs. C. Radouco-Thomas, F. Martin, S. Radouco-Thomas, G. Nosal and J. M. Chaumontet (Switzerland).

In earlier research we made a study in the rat of the relations between post-natal development of cerebral bioelectrical activity, behaviour and capacity for conditioning.[1] In the present work we examine the relations between electro-ontogenesis and certain aspects of the morphological and biochemical maturation of the rat cerebral cortex from birth to adult age.

The investigations were with longitudinal and horizontal series of W.A.G. rats at the age of 0, 4, 7, 10, 12, 14, 24, 30 and 90 days. The cerebral bioelectrical activity was defined by the spontaneous EEG and the strychnine potential. The EEG in ontogenesis seems to show a series of maturation stages towards the 4th, 7th and 14th days. Morphologically (Nissl, Weigert, Golgi-Cox preparations) the study of the ontogenesis of the cortical neurite was made from three angles: pericaryon, dendrite and axon. The quantitative evaluation of the morphological maturation of the neocortex is still being studied.

In research on cerebral metabolism, the consumption of O_2 by the brain of the young rat was examined in the post-natal period in relation to the critical moments of electro-ontogenic and structural maturation. Experimental cortical lesions (chemical decortication) were induced at various chronological ages and their effect studied in relation to electro-ontogenesis and capacity for conditioning. The action of nerve stimulants and depressants, as well as of ACTH, was examined in the spontaneous EEG and on the consumption of O_2 in the post-natal development of the rat brain.

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1. Radouco-Thomas, C., Martin, F., Chaumontet, J. M. and Royo, D. (1961), *Neuro-Psychopharmacology*, vol. 2, Elsevier, Amsterdam.

127 Relationship between Reserpine-Induced Facilitation of Evoked Potentials in the Limbic System and Change in Brain Serotonin Level. A. M. Revzin, S. Spector and E. Costa (U.S.A.).

Reserpine and a tetrabenazine analogue (Ro 4-1284) cause behavioural depression, depletion of brain 5HT and NE, and facilitation of electrically-evoked potentials in hippocampus. The latter effect is not induced by chlorpromazine, except in very large doses. Experiments were designed to see whether the effect on the limbic system is associated with the change in brain 5HT or the change in brain NE. In these studies the limbic potentials were measured in unanaesthetized free-moving cats with electrodes bilaterally implanted in amygdala and hippocampus.

Reserpine (1–3 mg/kg) or Ro 4-1284 (20–30 mg/kg) produced both behavioural depression and a facilitation of hippocampal potentials evoked by stimulation of the basolateral amygaloid complex. These doses of drugs also completely depleted brain of 5HT and NE. In contrast, 24 hr after administration of 50 mg/kg i.p. of α-MMT, a compound which selectively depletes NE, the brain NE was lowered by more than 80 per cent while 5HT levels were normal. This compound did not affect the limbic system potentials. If reserpine or the tetrabenazine analogues were administered 24 hr after 50 mg/kg i.p. of α-MMT, effects on the limbic system were the same as in cats given reserpine or Ro 4-1284 alone. These results indicate that electrophysiological as well as the behavioural effects of this tetrabenazine analogue and reserpine are associated with their action on brain 5HT binding sites and not those of NE.

128 Pharmacology of Hippocampus Pyramidal Cells. F. Brücke and C. Stumpf (Austria).

The hippocampus (HC) is a good test object for the study of pharmacological actions on central synapses since this region constitutes a simple type of cortex with its cell bodies and dendrites arranged in clearly distinct layers. Moreover, the HC EEG shows characteristic patterns: During an arousal reaction, a very regular 6 c/s rhythm ("Theta rhythm") occurs. The experiments were carried out in rabbits, the unit activity being recorded with steel microelectrodes. We were able to keep single cells for periods up to four hours.

Destruction of the Cajal nucleus in the median septum region or injection of procaine into that part of the septum abolishes the regular slow wave activity and leads to desynchronization which in the case of procaine disappears within 20 min. Intravenous injections of eserine but not of neostigmine induce analogous EEG changes as an arousal reaction and the pyramidal cells greatly increase their discharge frequency. Interruption of septal transmission blocks both the effects of eserine on the HC EEG and on the HC unit activity. Similar EEG changes are produced by amphetamine or methylamphetamine but these drugs do not increase the frequency of pyramidal cell firing although the EEG effect may again be abolished by septal lesions. Finally, nicotine also causes a HC theta rhythm during the first phase of its action accompanied by an increased firing rate of the pyramidal cells. Later, a hippocampus seizure pattern developes with inhibition of unit firing. The nicotine-induced theta rhythm but not the seizure pattern can be abolished by septal destruction. Therefore, it appears that any kind of HC EEG regularization is dependent on the integrity of the septum.

Electrical stimulation of the septum produces a variety of EEG responses in the HC dependent on the frequency of stimulation and on the localization of the stimulating electrodes. High frequency stimulation may lead to an EEG desynchronization or to a theta rhythm whereas low frequency stimulation may produce a HC response which is similar in appearance to the cortical recruiting response. This latter response is inhibited by LSD.

LSD, although inducing similar neocortical EEG changes to eserine, amphetamine or an arousal reaction, has quite a different effect on the HC activity. It produces a flattening of the HC EEG and decreases or abolishes hippocampal unit firing for periods of time up to 2 hr.

129a Neuro-psycho-pharmacologie stéréotaxique—Analyse électrographique et action neuro-végétative du N-N¹ diéthylamide de l'acide 2-méthoxy-4-allylphénoxyacétique pendant des explorations fonctionelles stéréotaxiques chez l'homme. J. Bancaud, M. Rebufat-Deschamps, J. Thuillier and P. Deligne (France).

A l'occasion d'explorations fonctionnelles stéréotaxiques, pratiquées chez l'homme dans des buts thérapeutiques précis, on a analysé l'action centrale du N-N¹-diéthylamide de l'acide 2-méthoxy-4-allylphénoxyacétique (G.29505).

Cette substance, qui possède à la fois une action narcotique puissante et des propiétés analeptiques respiratoires, a été étudiée corrélativement au point de vue de ses incidences sur l'activité électrique cérébrale et sur certaines régulations végétatives.

L'enregistrement simultané de nombreuses formations centrales (cortex, sous-cortex, noyaux gris centraux) a été réalisé par implantation aigue ou chronique d'aiguilles à contacts étagés (100–300 électrodes intra-cérébrales) chez des malades présentant des syndromes divers (épilepsie, douleurs, mouvements anormaux, etc.).

Les résultats, analysés en fonction des modalités d'administration et de durée d'injection du N-N_1 diéthylamide de l'acide 2-méthoxy-4-allylphénoxyacétique, ont mis en évidence les possibilités de dissocier son action narcotique de ses effets respiratoires et cardiaques. D'autre part, ses points d'impact sur diverses structures cérébrales ont été définis, et permettent d'envisager son utilisation comme révélateur de l'origine de certaines manifestations paroxystique initiales.

Ainsi, cette première réalisation d'une étude de neuro-psycho-pharmacologie stéréotaxique chez l'homme parait ouvrir des perspectives particulièrement intéressantes en ce qui concerne l'analyse des modalités d'action de certaines drogues sur le système nerveux central.

129b Stereotaxic Neuro - Psycho - Pharmacology - Electrographic Analysis and Neurovegetative Action of the N-N'-Diethylamide of the 2 - Methoxy - Allylphenoxyacetic Acid (G.29505) during Stereotaxis Functional Investigations in Man. J. Bancaud, M. Rebufat-Deschamps, J. Thuillier and P. Deligne (France).

During functional stereotaxis investigations on man designed to evolve effective treatment, the central action of the N-N^1-diethylamide of 2-methoxy-4-allylphenoxyacetic acid (G.29505) was analysed.

This substance, which possesses powerful narcotic and analeptic respiratory properties, has been studied from the point of view of its effect on the electrical activity of the brain and on certain forms of vegetative regulation.

Simultaneous recording from several central structures (cortex, sub-cortex, central grey nuclei) were carried out by acute or chronic implantation of needles with graded contacts (100–300 intracerebral electrodes) on patients with various syndromes (epilepsy, pain, abnormal movements, etc.).

The results, analysed with devised variations in administration and duration of the injection of the N-N_1 diethylamide of 2-methoxy-4-allylphenoxyacetic acid, have shown the possibility of dissociating its narcotic action from its respiratory and cardiac effects.

In addition, the points of its impact on various cerebral structures have been defined and indicate its possible usefulness in revealing the origin of certain paroxysmal manifestations.

Thus, this first stereotaxic psycho-pharmacological study to be carried out in man appears to suggest interesting prospects in regard to the analysis of the mechanism of action of certain drugs on the central nervous system.

130 Metabolism Underlying the Electrically Stimulated Separated Cerebral Cortical Tissues. S. Kozawa (Japan).

When adequate electrical stimulation is applied to separated cerebral cortical tissues, there occurs an increase in the tissue respiration and inorganic phosphate, and this phenomenon is characterized by a distinct substrate specificity. Such specific substrates coincide with those substrates which are capable of restoring the insulin-induced hypoglycaemic EEG temporarily to the normal, when applied topically to the cortical surface, and which are capable of accelerating the ACh formation in cortical slices. Moreover, destruction of the cell structure seems to destroy considerably the mechanism upon which this phenomenon rests.

The electrical stimulation of cerebral slices increases the tissue respiration and glucose uptake and accelerates its glycolysis. In non-stimulated slices the effect of narcotics is similar to that of the stimulation, except for the depression of respiration. On the contrary, in electrically stimulated slices, narcotics practically abolish all the changes produced by the electrical stimulation, and moreover, this effect is striking at low concentrations where narcotics are no longer active on the non-stimulated slices. Characteristically, the pattern of metabolic changes in the brain under narcosis coincides with that of the electrically stimulated cerebral cortical slices produced by narcotics.

According to the author's study, the metabolism in slices provoked by the electrical stimulation, appears to proceed in conformity with Warburg–Dickens' scheme, judging from the viewpoint of carbohydrate degradation; it may be that this metabolic process is closely linked with the nervous activity.

131 On the Localization of the Action of Aminazine and Analgesics in the Reticular Formation of the Brain-Stem. A. V. Valdman (U.S.S.R.).

Structures of the reticular formation (R.F.) sensitive to aminazine and analgesics were located by means of pin-point unipolar stimulation of various areas of the brain-stem in the cat (with determination of electrodes' localization) and recording of the response. Spontaneous and evoked activity of separate functional units of R.F. was recorded extracellularly by a capillary microelectrode.

Aminazine depresses the descending (alleviating or inhibiting) influences on the spinal reflexes and vascular (pressor or depressor) responses caused by stimution of the hypothalamic nuclei, of the vestibular complex, and of the upper segments of the reticulo-spinal tract. It exerts little influence on these responses and reflexes when stimulation is applied to the red nuclei, the tegmento-olivary and the tecto-spinal tracts, the nucleus of the spinal branch of the trigeminal nerve, the dorsal nucleus of the vagus and certain reticular nuclei of the medulla.

The analgesics depress the descending inhibiting influences coming from the nuclear structures of R.F. (the reticular nuclei of the medulla and pons, the spinal branch of the trigeminal nerve, the dorsal nucleus of the vagus) but have little effect on the results of stimulation of the descending tracts (vestibulo- and tecto-spinal tracts, the solitary and unciform bundles).

Effects of stimulation of R.F. of the brain-stem caused by afferent impulses (activization response in the EEG after painful, light, sound and interoceptive stimulation; reflex changes of the blood pressure and respiration following stimulation of various receptor zones) were modified by the analgesics and aminazine differently, depending on the morphological peculiarities of the afferent pathways.

On the facts obtained the author gives a schematic drawing demonstrating the localization of action of aminazine and analgesics in the reticular formation of the brain-stem.

132 On the Relationship of Bioelectric Activity of the Central Nervous System to Metabolism. V. V. Vasilyeva (U.S.S.R.).

Up to the present time the problem of the role of metabolism in the origin of biopotentials is still under discussion. For a number of years the staff of our department has been carrying out studi ±o to reveal any dependence of bioelectric activity of the cortex and subcortex upon the functional state of the central nervous system, which is determined by the level of conditioned reflex activity and metabolism. The latter was determined by absorption of oxygen in the tissues of the brain, excretion of carbon dioxide and contents of pyruvic acid. The intensity of protein metabolism was determined by the amount of non-protein nitrogen, the activity of cathepsins and the degree of inclusion of sulphur-35 methionine. Adenosine triphosphoric acid is regarded as a connecting link between processes of liberation of energy and metabolism that is a specific biochemical feature of the nervous tissue activity. With the help of labelled phosphorus-32 we have studied qualitative indices of acid soluble phosphoric fractions and the velocity of restoration. Various pharmacological substances were used as analysers.

For example, it was found that echinopsin increases the bioelectric activity of the cortex. This is accompanied by a decrease of adenosine triphosphate and phosphocreatine in the tissues and by a simultaneous increase of inorganic phosphorus. The intensity of restoration of adenosine triphosphate is retarded, but that of the phospholipids, phosphoproteins as well as desoxyribonucleic acid is increased. At the same time absorption of oxygen is increased. Echinopsin is able to change the distribution and the velocity of excretion of sulphur-35 thiopental or radioactive products of its transformation. In intoxication caused by botulism the amplitude of biopotentials of the cortex is decreased, the resynthesis of creatine phosphate and adenosine triphosphoric acid is retarded and the process of decarboxylation of ketonic acids in the tissues of the central nervous system is disturbed. Using doses of of ACTH that provide an increase of conditioned reflex activity, the intensity of absorption of oxygen is increased and the bioelectric potentials are activated. Large doses of ACTH give opposite results.

Ganglion-blocking substances create a prevalence of inhibitory processes that affect the resynthesis of proteins. This may be seen in a decrease of absorption of oxygen and sulphur-35 methionine by the proteins of the brain tissues. When using local anaesthetic substances, we found that the increase of the inhibitory processes is accompanied by a decrease of absorption of oxygen by the brain tissues. Papaverine inhibits biopotentials and decreases metabolism.

It may be concluded that changes in biopotentials are closely related to the shifts in metabolism.

133 An Analysis of Tranquilizers in Chronically Electrodes-Implanted Cat. R. Kido and K. Yamamoto (Japan).

Experiments were performed to analyze the characteristics of tranquilizers and barbiturates, from both the electrical activity of the brain and the behaviour in chronically electrodes-implanted cats.

(1) After intravenous injection of chlorpromazine (1–15 mg/kg) or reserpine (0·1–1·0 mg/kg), as seen from behaviour and brain electrical activity, it is difficult to cause arousal reactions by mechanical or auditory stimulation; however, chlorpromazine usually causes drowsy to rest patterns in the neocortex and rarely deep-sleep patterns. After reserpine administration, in contrast to the hippocampus and amygdala, the neo-cortex does not show any deep-sleep pattern.

(2) Phenobarbital (20–100 mg/kg) and pentobarbital (10–30 mg/kg) intravenously, causes sedatation, sleep and narcosis. EEG shows mostly high-voltage slow waves in the sensory area and the thalamus, multiple spikes in the amygdala, and low-voltage fast waves in the hippocampus. However, the amygdala and hippocampus recover relatively rapidly.

(3) Meprobamate (30–120 mg/kg) intravenously causes the appearance of 14–20 c/s rhythmical waves with a gradual increase of slow waves in the auditory area and thalamus. When these slow waves are appearing, spike discharges are often seen in the amygdala. Characteristic 25 c/s high-voltage waves are seen in the hippocampus at higher doses of meprobamate, and the consciousness level from the behaviour is drowsy. Further experiments in chronically electrodes-implanted dogs are in progress and comparative observatives will be discussed.

134 The Effect of Mescaline on the Optic Evoked Potential. J. R. Smythies, W. P. Koella and C. K. Levy (U.S.A.).

Rabbits were prepared with indwelling silver wire electrodes resting on the optic cortex. Without anaesthesia the optic evoked potential consisted of a primary biphasic surface positive-negative wave followed by a series of fast waves superimposed on a slower biphasic swing. These responses were photographed and series of these individual waves were measured and the results subjected to statistical analysis. Mescaline in doses of 5, 10, 20 and 40 mg/kg was injected intravenously and its effect on these waves was determined as well as the effect of saline controls. At low doses, the drug causes potentiation of the waves and at higher doses an initial period of inhibition appears. The dosage level where this inhibition appears and its degree

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differs for each wave. With wave 1, potentiation is dominant and inhibition only appears at 20 mg/kg. In the case of wave 2, the inhibition appears at 10 mg/kg and lasts twice as long at 20 mg/kg. For waves 3 and 4, the inhibitory phase is dominant and potentiation hardly appears except at the lowest dose level. Changes in the latency of the waves is also reported. On a basis of these results it was possible to draw satisfactory dose–response curves so that this test may be used in a quantitative measure of drug activity.

136 Kinetics of Rifomycin SV in Body Fluids. E. Beccari, G. Bianchi and G. Maffii (Italy).

The blood levels of the new antibiotic, rifomycin SV, given intravenously to dogs, as well as the amounts found in bile and urine, were followed for 7–8 hr after administration. Single doses of 10 mg/kg and single and repeated doses of 5 mg/kg were used, the time intervals between two subsequent injections being 210 min. The samples were assayed microbiologically, employing a disk plate method using *Sarcina lutea* as the test organism.

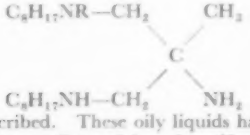
The blood–concentration curves plotted as log µm/ml against time showed four components which may be accounted for by assuming a distribution of the drug into two or three compartments in which linear and saturated processes occur.

The total and fractional amounts of rifomycin SV excreted in urine are 6–8 times lower than those found in the vile collected in the same time; excretion of the drug in the latter seems to be a saturated process, whereas this is not the case in renal excretion.

The transfer of rifomycin SV in the systems interposed between blood and bile and urine has been analysed by deconvoluting the biliary and urinary amounts against the corresponding blood levels, and the inherent kinetics has been determined.

137 New Broad-Spectrum Antimicrobial Triamine Synthetic Compounds. M. G. Mulinos (U.S.A.).

New antimicrobial compounds belonging to the series:



will be described. These oily liquids have proved to be potent antibacterial and antifungal agents when applied topically against a wide range of organisms, although they are relatively ineffective against *Candida*. Strongly substantive to both natural and synthetic textile fibres, they protect these against deterioration by micro-organisms. They are also strongly deterrent to mildew and wood rot, and render wool resistant to moth damage.

The antimicrobial effects of the triamines will be correlated with their chemical structure and solubility in protoplasm. Data will be presented on oral and parenteral toxicity, and on tissue distribution as determined by a ^{14}C tagged compound. Oral LD_{50} was 0·8–1·0 g/kg; intraperitoneal LD_{50} was 90–100 mg/kg in the rat. Chronic oral administration showed no cumulative toxicity.

These alkaloids are insoluble in water; they form soaps with fatty acids and water-soluble salts with organic and mineral acids. Their wide antimicrobial activity is ascribed to the fine balance between their lipophilic and hydrophilic properties which allow penetration of the cell membrane. The compounds are stable in suitable vehicles and do not readily become detoxified in contact with the tissues. They were found to be non-irritating and non-allergenic to human skin by the usual tests. They have proved effective clinically in pyodermias and fungous infestations of the skin, and are finding a wide range of industrial uses.

138 Structure–Activity Relationships in New Penicillins. I. M. Rollo and G. F. Somers (United Kingdom).

These studies are a result of the search for new penicillins with improved properties over those of existing penicillins. Initially, the study was restricted by the limitations of the biosynthetic techniques. Latterly, the use of 6-aminopenicillinic acid has resulted in the ready preparation of new molecular species. Properties looked for included increased acid stability, improved intestinal absorption, improved antibacterial spectra and penicillinase resistance.

Testing methods included routine sensitivity tests using a range of human pathogens *in vitro* and conventional blood level determinations in laboratory animals and human volunteers. Interpretation of the effectiveness of orally administered compounds compared with subcutaneous administration in mice infected with *Streptococcus pyogenes* has proved valuable in the selection of orally active penicillins for human volunteer trial. A 24-hr test was devised for the determination of anti-staphylococcal activity using mice infected with normal and penicillinase-producing strains of *Staphylococcus aureus*.

Penicillins were made with side chains representing six different types of molecular structure, several representatives being included in each group. Between groups there was considerable variation in antibacterial activity, resistance to penicillinase, and sensitivity to acids. Major variations also occurred in the blood levels obtained and the degree of absorption from the gut. In certain cases the presence of serum caused a marked fall in antibacterial activity.

Differences between groups of penicillins had enough significance to permit a forecast of the properties of other penicillins in each group, and in a number of cases the estimate was confirmed by the preparation of further penicillins.

139 Correlation of Chemical Structure of the Penicillins and Biological Activity. H. G. Steinman (U.S.A.).

Several new penicillins have been investigated for antimicrobial activity, as substrates of penicillinase, and as inducers of penicillinase. These studies demonstrated that a small degree of activity was retained in 6-aminopenicillanic acid, the nucleus of penicillin without an acyl side chain. However, an acyl side chain was essential for full biological activity and conferred specificity to both the degree of antimicrobial activity and the range of the antibacterial spectrum. A change in steric configuration of the side chain was found to modify the antibiotic activity of the molecule. A variety of substituents exerted moderate but specific effects on the rate of reaction with penicillinase. Stereo-isomers were hydrolysed at different rates. Moreover, they responded very differently to two different penicillinases. A striking change was produced when the aliphatic amide linkage common to the conventional penicillins was replaced by an aromatic amide grouping. The resultant molecule (2:6-dimethoxyphenylpenicillin) was highly resistant to the action of penicillinase. A number of competitive interactions were observed. The D-isomer of α-phenoxyethylphenylpenicillin inhibited the hydrolysis of the L-isomer by staphylococcal penicillinase; 2:6-dimethoxyphenylpenicillin inhibited hydrolysis of benzylpenicillin. Induced formation of penicillinase, a response specific for the penicillin structure, was much less affected by the nature of the side chain. In the case of 2-6-dimethoxyphenylpenicillin, persistence of the inducer against the destructive action of the enzyme played an important role. The unnatural L-configuration of the thiazolidine carbon atom adjacent to the carboxy radical rendered the molecule devoid of antibiotic activity and incapable of acting as a substrate for penicillinase.

140 Elevation of Penicillin Serum Concentration by Sulphamethoxipyridazine. J. Sarmiento and G. Montes (Colombia).

Triple sulphonamides can reduce the renal excretion of penicillin. However, Bayne *et al.* disproved that effect. Nothing has been said about this effect, so far as we know, in relation with the slowly-excreted sulphamethoxipiridazine.

To study this the following experiments were designed: Dogs, weighing between 7 and 11 kg, were divided into three groups. Serum penicillin activity was measured during 3 hr following the intravenous application of 10,000 units per kg. The second group, besides penicillin, received half an hour before 10 mg/kg of sulphamethoxipiridazine intravenously. The third group was nephrectomized bilaterally, before penicillin.

For comparison purposes we took serum penicillin activity 1 hr after application, as the 100 per cent value and observed it for 2 hr more. The results were expressed as the residual percentage of activity.

In the group treated with penicillin alone the remaining serum penicillin activity after 3 hr was 15·63 per cent, standard error: 3·35. In the second group (penicillin plus sulphamethoxipiridazine) the remaining serum penicillin activity was 32·69 per cent, standard error: 4·81. In the nephrectomized group the remaining activity was 87·5 per cent, standard error: 12·5 (four animals only).

Statistical analysis shows the level of significance between first and second group is $P < 0{\cdot}01$; between the second and third group, $P < 0{\cdot}001$; and between first and third group $P < 0{\cdot}001$.

At the moment experiments are in progress in order to measure simultaneously the glomerular filtration rate.

141 The Relation between Duration of Action of Sulphonamide Compounds and Their Clearance. Y. Minesita and K. Yamamoto (Japan).

A sulphonamide compound, 5-methyl-3-sulphonilamido-*iso*oxazole (Sulfisemezole) is known as a long acting drug, while its very near compound in chemical structure, 3:4-dimethyl-5-sulphanilamido-*iso*oxazole (Sulfisoxazole) is rather short acting. Metabolic properties of these two drugs are studied together with several other long acting sulpha drugs.

No difference in the absorption from the alimentary canal was found between sulfisomezole and sulfisoxazle. The amount of the drug distributed in the body tissue was not so high as to make clear the mode of long action. The interaction of the sulpha drugs with serum protein does not always parallel drug maintenance in the blood.

The renal excretion of the drugs is responsible for the long maintenance in the blood. The clearance test in dogs showed that the clearance value of the long acting sulphonamide compounds, such as sulfisomezole, sulfadimethoxine and sulfamethoxypyridazine was 1/2 to 1/4 of the glomeruler filtration rate, indicating remarkable tubular reabsorption, whereas that of fulfisoxazole, sulfisomedine and other short acting sulpha drugs, as well as N-4-acetyl sulphisomezole was 3 to 4 times higher than the glomeruler filtration rate indicating active tubular excretion.

142 The Metabolism of some Sulphonamides in regard to the Duration of their Action in Experimental Animals. C. Bertazzoli, T. Chieli and C. Ciceri (Italy).

A new long-acting sulphonamide, 2-*p*-aminobenzensulphonamido-3-methoxy-pirazine, has been synthesized in our laboratory. Since the sulphonamides with analogous characteristics have been stimulating widespread interest, we have studied thoroughly the pharmacology of our drug and of other sulphonamides, both with short- and long-term action, with the aim of making clear the mech-

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anism by which they remain in the host organism for a long period of time.

Our investigation was carried out in experimental animals and we studied the behaviour of blood levels, tissue and body fluid concentrations, plasma protein bound, renal clearance and urinary metabolites.

The data obtained indicated some aspects of the metabolic behaviour of these substances in regard to the duration of their action.

143 Effect of Isoniazid on the Production of Tuberculous Immunity. F. M. Sullivan (United Kingdom).

As part of the anti-tuberculosis campaign in backward countries, it has been suggested that BCG vaccination and INH prophylactic treatment should be given to children simultaneously. We have investigated the effect of isoniazid on anti-tuberculous vaccination using a variety of vaccines both virulent and avirulent.

In mice, a small vaccinating dose of either virulent (multiplying) or avirulent (non-multiplying) bacilli will confer good immunity to a subsequent challenge. If a small vaccinating dose of virulent bacilli are inhibited by INH during the vaccin tion period, then no immunity is produced. That this is not due merely to the inadequate number of bacilli, in the vaccine, is shown by the fact that a very large vaccinating dose of virulent bacilli (more than the final number achieved after multiplication of the small vaccinating dose without treatment) when suppressed by INH does not confer immunity. Further, the avirulent strain of tubercle bacillus H37Ra will confer immunity without multiplication of the vaccine, but will not do so when suppressed by INH. The effect of INH on INH-resistant BCG is at present being studied.

144a Relations entre la Fixation Tissulaire et la Durée d'Action des Trypanocides. F. Benazet (France).

A l'aide d'une méthode biologique originale de dosage, l'auteur étudie la répartition dans l'organisme du rat de quelques produits (phénanthridines, pentamidine) qui, injectés à l'homme et aux animaus domestique, manifestent une activité préventive de longue durée vis-à-vis des trypanosomes.

Après administration sous-cutanée unique, ces produits ne sont pas décelables dans le sang du rat ou ne le sont que pendant une très brève période, alors qu'ils sont trouvés en quantité appréciable dans certains organes (foie, rate) pendant plusieurs semaines ou plusieurs mois.

Leur métabolisme est bien différent de celui des médicaments qui manifestent une bonne activité curative sur les trypanosomes mais sont dépourvus d'activité préventive. En effet, ceux-ci déterminent des concentrations sanguines qui peuvent êtres élevées, mais ils sont rapidement éliminés de l'organisme.

La comparaison de diverses phénanthridines montre un parallélisme entre la durée de la protection qu'elles confèrent à l'animal et leur persistance dans les organes.

La localisation tissulaire des trypanocides dans les organes et le mécanisme de l'action antiparasitaire du produit fixé sont discutés.

Après une revue des divers facteurs qui conditionnent la durée d'action des trypanocides préventifs, l'auteur conclue à l'importance de leur fixation sur certains tissus.

144b Relationship of the Fixation of Trypanocides in Certain Tissues to the Duration of their Action. F. Benazet (France).

The author has studied the distribution of certain substances (phenanthridine, pentamidine) in the body of the rat by means of an original biological dosage method; these substances, when injected into man or domestic animals, show long-lasting preventive effects against trypanosomes.

After a single subcutaneous injection these drugs are either not detectable in the blood of the rat or can be found for a very brief period, while they are found in appreciable amounts in certain organs (liver, spleen) for several weeks or months.

Their metabolism is very different from that of drugs which manifest a good curative effect on trypanosomes but are without preventive effect. In fact, these may attain high concentrations in the blood but are rapidly eliminated by the body.

Comparison of various phenanthridines shows a parallel between the duration of the protection conferred by them on the animal and their persistence in the organism.

The localization of the trypanocides in the organs and the mechanism of the antiparasitic action of a particular drug are discussed.

Following a survey of various factors which affect the duration of the action of preventive trypanocides, the author comes to the conclusion that their fixation in certain organs is important.

145 Mechanism of the Selective Virus Inhibitory Action of 2-(α-Hydroxybenzyl)-benzimidazole. H. J. Eggers and I. Tamm (U.S.A.).

2-(α-Hydroxybenzyl)-benzimidazole (HBB) has been shown to inhibit the cytopathological effects of 34 different enteroviruses, but not those of 15 viruses belonging to other groups. The virus inhibitory action was independent of the cell system used, since it was demonstrated in primary monkey kidney cell cultures as well as in two continuous culture cell lines (HeLa and ERK). At virus inhibitory concentrations (20–200 μM), the compound caused no, or only slight changes in the morphologic appearance of cells, and it did not interfere with the following cellular metabolic activities: oxygen consumption,

glucose utilization, lactic acid production, incorporation of adenosine into RNA, or of alanine into protein. Furthermore, the compound did not significantly alter the rates of multiplication of host cells.

Strong evidence was accumulated that HBB interferes with some intracellular step in virus multiplication, and thereby inhibits virus-induced cell damage. The compound did not affect adsorption of HBB-susceptible viruses to host cells, but was shown to act at a late stage in the reproductive cycle of enteroviruses. However, effects during the early intracellular stages have not been excluded. Synthesis of infective viral nucleic acid and of viral antigens in the presence of HBB are under investigation. The compound had no direct inactivating effect on the infectivity of susceptible viruses. The available evidence suggests that HBB inhibits a step of specific and decisive importance in the reproduction of enteroviruses. The relationship between the structure of HBB and its selective virus inhibitory action will be discussed

146 Is Urinary 5-Hydroxytryptamine Formed in the Kidney? M. M. Airaksinen and V. J. Uuspää (Finland).

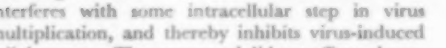
Vane's rat fundus method proved useful for determining 5-hydroxytryptamine (5HT) in unextracted rat urine. In hydrated rats, 5HT (1 mg/kg i.p.) increased 5HT and 5-hydroxyindoleacetic acid (5HIAA) excretion eight- and six-fold respectively. Of the injected 5HT, 0·6 per cent was excreted unchanged and 35 per cent as 5HIAA. After 5-hydroxytryptophan (5HTP), 0·05–50 mg/kg of DL-form i.p., the excretion of 5HT was ten- to 5000-fold. Thirty-eight point six per cent of the L-5HTP was found in the urine as 5HT and after large doses, 24·4 per cent as 5HIAA. The 5HT content of the kidney increased ten-fold within an hour after 5HTP (5 mg/kg i.v.). The 5HT content of the blood in renal vein was elevated and higher than that of the blood in the abdominal aorta. This difference was found even before 5HTP injection. Reserpine (5 mg/kg i.p.) given 48 hr earlier did not modify the conversion of 5HTP to 5HT and 5HIAA. Antidiuresis occurred during 2 hr after intraperitoneal administration of 2–5 mg/kg of 5HT, but not after 5HTP.

When rat kidneys were perfused *in situ*, the addition of 5HTP to Locke solution was followed by the abundant appearance of 5HT in the filtrate collected from the bladder and also in the perfusate from the inferior vena cava.

Thus the kidney is able to synthesize appreciable amounts of 5HT, which it transfers to the blood and urine and this also seems to take place physiologically.

147 The Metabolism of Dehydroacetic Acid. T. E. Barman, D. V. Parke and R. T. Williams (United Kingdom).

Dehydroacetic acid (DHA; 3-acetyl-6-methyl-2:3-dihydropyran-2:4-dione),



is a fungicide [^{14}C]DHA labelled in 4 of its carbon atoms was synthesized from $CH_3{}^{14}CO.Br$. When the labelled compound is fed to rats in small doses (60 mg/kg), the excretion of ^{14}C in the urine is slow. In 5 days, the distribution of ^{14}C in 3 animals was found to be: urine 23 per cent, faeces 19 per cent, respiratory CO_2 12·4 per cent, and carcase 22 per cent. This slow excretion is in keeping with its known ability to bind with proteins. The urine contained five radioactive compounds: unchanged DHA (4·7 per cent of the dose), hydroxy-DHA (7 per cent), triacetic acid lactone (1·2 per cent), urea (0·2 per cent) and an unknown metabolite still containing the pyrone ring. Since DHA and hydroxy-DHA readily combine with ammonia in dilute solution to form imino compounds, imino-DHA (3-acetimidoyl-6-methyl-2:3-dihydropyran-2:4-dione) and imino-hydroxy-DHA were often found as artifacts in the urine and interfered with isotope dilution procedures for DHA and hydroxy-DHA. The hydroxy-DHA was isolated (m.p. 133°) and from its reactions it is believed to be 3-glycolloyl-6-methyl-2:3-dihydropyran-2:4-dione.

Our experiments suggest that DHA is metabolized first by oxidation of the 3-$COCH_3$ to a -$CO.CH_2OH$ group. The hydroxy-DHA is then oxidized to triacetic lactone (TAL) and CO_2. Further metabolism of TAL would result in the formation of triacetic acid which is known to be readily metabolized by tissues *via* acetoacetic and acetic acids.

^{14}C-Triacetic acid lactone was prepared by deacetylation of ^{14}C-DHA. When the TAL was fed to rats (350 mg/kg), it was rapidly excreted, 18 per cent being excreted in the urine in one day, and 45 per cent of the ^{14}C appearing in the respiratory CO_2. In 3 days after dosing, 46 per cent of the ^{14}C appeared as respiratory CO_2, 20 per cent appeared in the urine almost entirely as unchanged TAL (19·6 per cent TAL and 0·3 per cent urea), 6 per cent in the faeces and 13 per cent in the carcase.

(This work was supported by a grant from the Distillers' Company Limited.)

148 Metabolic Fate of Phenothiazines. J. V. Kinross-Wright and J. B. Ragland (U.S.A.).

As a first step in these studies we have established a spectrofluorimetric method for the determination of pheno thiazines in biological fluids and tissues. The sensitivity of the method is sufficient to enable us to carry out drug uptake and the distribution studies that have heretofore only been possible using radioisotopes.

Using this method, the blood levels of several phenothiazines have been determined in subjects

given a single oral dose of the drug. These blood level data will be presented along with urinary excretion data.

Thioridiazine has been determined in the tissues of rats following intraperitoneal injection. All tissues examined are able to concentrate the drug in less than two min after injection. Studies are now in progress to determine the distribution of the drug within regions of the brain and other tissues. The intracellular concentration of the drug is also to be studied through the use of precipitation and ultracentrifugal techniques to fractionate the cellular constituents.

149 Comparative Studies on Sulphanilamide Acetylation; an Inhibitor in Dog Liver. K. C. Leibman and A. M. Anaclerio (U.S.A.).

The absence of acetylation of sulphonamides and other arylamines in dogs has been long recognized. We have found that dog liver slices hydrolyzed acetylsulphanilamide more rapidly than did rabbit liver slices, but did not acetylate sulphanilamide under conditions where acetylation was catalyzed by rabbit liver slices or pigeon liver homogenates. Incubation of [^{14}C]acetate with unlabelled acetylsulphanilamide and sulphanilamide caused no accumulation of radioactivity in acetylsulphanilamide in the presence of dog liver slices, but an extensive acetate exchange took place with rabbit liver slices. Dog liver acetone powder extracts promoted the acetylation of glucosamine to a greater extent than did those from pigeon liver. These results suggest that the lack of acetylation of arylamines in dogs is not due to an unfavourable acetylation–deacetylation equilibrium, nor to a defect in acetylcoenzyme A formation, but rather to a lack of active arylamine acetylase. Dog liver homogenates or acetone powder extracts reversibly inhibited the acetylation of sulphanilamide in various pigeon liver preparations. The inhibitor was thermolabile, non-dialyzable, and was deactivated by protein precipitants, but survived lyophilization; it thus appears to be closely associated with protein. It did not inhibit the acetylation of glucosamine in pigeon liver, nor did it catalyze the hydrolysis of acetylsulphanilamide. Dog liver acetone powder extracts did not catalyze transacetylation between acetylsulphanilamide and aminoazobenzenesulphonic acid, a reaction which was demonstrated in pigeon liver acetone powder extracts. Dog liver preparations inhibited this pigeon liver arylamine transacetylase system.

150 Factors Influencing Induction of Microsomal Drug-Metabolizing Enzymes of Liver. R. Kato, P. Vassanelli and E. Chiesara (Italy).

At the present time it is very well known that some drugs (inducing drugs) can modify the activity of some hepatic microsomal enzymes responsible for the breakdown of other drugs(induced drugs). As the mechanism by which the phenomenon occurs is not yet known, we have performed some investigations in order to analyse the influence of particular experimental conditions on the phenomenon.

In the work reported here phenobarbital and phenoglycodol were used as inducing drugs; pentobarbital and meprobamate as induced drugs. The modifications in the pharmacological effect of induced drugs were taken into account as well as the modifications in their catabolism. In fact, concentration curves of the drugs in the induced animals were determined and also *in vitro* metabolism of the drugs was carried out. By varying the experimental conditions the influence on the induction was examined: (1) modification of protein and nuclei acid biosynthesis, by means of fasting, administration of hypo- and hyperprotein diets, ethionine, 6-mercaptopurine, 8-azaguanine and radiations; (2) modification of liver function and particularly the activity of the reticulo-endotelial system, by means of carbon tetrachloride, trypan blue, carbon black, orotic acid, glucuronic acid, ascorbic acid or partial hepatectomy or splenectomy; and (3) modification of hormonal regulation, by means of hypophysectomy, thyroidectomy, adrenalectomy or castration. Furthermore, the effects of simultaneous administration of the following with inducing drugs were studied: (a) drugs which can induce a variation of the metabolism of zoxazolamine, but do not modify the metabolism of phenobarbital and meprobamate (3:4-benzpyrene and methylcholantrene); (b) drugs have a chemical structure similar to that of the inducing drugs, but which do not cause any inducing effect (megimide, phthalglutarimide, barbituric acid, uracil); and drugs antagonizing the pharmacological effect of the inducing drugs (amphetamine, coramine, ritaline). The results of each group of experiments are described and their meanings are discussed.

151 Increase of Rhodanase Activity by Dimercaptopropanesulphonate Sodium (DMPS). I. P. Lapin (U.S.S.R.).

The prophylactic or therapeutic use of DMPS (orally, subcutaneously, intraperitoneally) in doses of 160–800 /g (2–20 times higher than the cyanide doses in molecular ratio) diminished the mortality in adult mice and rats 20–90 per cent (500 animals). Its toxicity was significantly less than that of thiosulphate, but more than that of BAL.

Combined treatment with small doses of thiosulphate and DMPS (each dose was ineffective separately) significantly increased the protective action of the antidoes (mortality in mice decreasing from 80 per cent ± 10·0 to 30 per cent ± 15·3).

In the following experiments the role of DMPS in enzymatic rhodan-formation was ascertained. DMPS significantly increased the urinary excretion of SCN in rats poisoned with NaCN (67 animals). In experiments *in vitro* with rat liver preparations prepared according to Lang's technique, it was observed (142 samples) that DMPS in concentration of 10^{-5}–10^{-10} M increases the conversion of CN to

SCN by 1½–2 times when thiosulphate as a sulphur donor is in the medium.

Tetrasulphide (oxidized DMPS), a substance devoid of SH-groups and containing two disulphide bonds, has no effect on the enzymatic rhodan-formation *in vitro*. The role of SH-groups in rhodanase activation and the use of SH-agents as rhodanase activators in combination with sulphur donators in the treatment of cyanide poisoning are discussed.

152 Possible Role of Enzyme Inhibition and Repression in Drug Tolerance and Addiction. D. B. Goldstein and A. Goldstein (U.S.A.).

Tolerance and addiction may reflect changes in the rate of synthesis of enzymes whose substrates or products affect the brain. We propose that an addicting drug may inhibit an enzyme whose synthesis is repressed by its product. Enzyme inhibition accounts for the immediate drug effect. Tolerance develops because low concentrations of the product lead to increased enzyme synthesis. Thus the tolerant or addicted animal has an increased amount of enzyme, balanced by the drug inhibition. On withdrawal, the inhibition is rapidly relieved and the excess enzyme causes symptoms generally opposite to the initial drug effects. Repression resumes, however, enzyme levels slowly return to normal, and the physiological homeostasis is re-established.

Barbital (0·02 M) inhibits growth of *E. coli* B and affects its aminoacid metabolism. The Qo_2 of washed intact cells oxidizing a single aminoacid was measured with and without barbital to test the primary drug effect. Cells grown in the presence of barbital showed the long-term changes. In some cases, these effects were in opposite directions, e.g. alanine oxidation was inhibited by barbital, but was abnormally rapid in barbital-grown cells. This may represent de-repression of an inhibited enzyme, as predicted by the hypothesis.

Several aminoacid derivatives are thought to play a role in the brain, e.g. γ-aminobutyric acid, 5-hydroxytryptamine and the catecholamines. Altered aminoacid metabolism is therefore a possible mode of action of barbiturates which might warrant further study with brain tissue.

153 Influence of Sex Difference on the Pharmacological Action and Catabolism of Some Drugs. R. Kato, E. Chiesara and G. Fontino (Italy).

It was previously demonstrated that some barbiturates (Holck *et al.* (1937)) exert a different intensity of effect in the rat, according to sex. Such differences might be ascribed to the male rat's capacity of catabolizing barbiturates more easily in comparison with female rats (Crevier *et al.* 1950).

We have observed that the variations in pharmacological effects due to sex difference are to be seen in drugs which are degraded in the organism by hepatic microsomal enzymes, requiring the presence of TPNH and oxygen. The above-mentioned microsomal enzymes are inhibited by SKF-525A, marsilid and INH. They have another common characteristic: that of being increased in the animals that has undergone (48 hr before) a treatment with particular "inducing" drugs: phenobarbital, phenaglycodol, etc. We paid particular attention to the catabolism of pentobarbital, carisoprodol, strychnine, picrotoxin, OMPA in male and female rats.

It was observed that the differences in pharmacological effect and catabolism according to sex, are to be seen only in adult rats, not in immature ones.

The catabolism in male rats is generally about 2·5 times greater than in female rats.

The male hormones and 4-chlortestosterone can enhance the catabolism of drugs in castrated female rats to 70–80 per cent of the male rats' catabolism.

The castrated males show a diminished catabolism.

In guinea pigs and mice no change in the catabolism of the above-mentioned drugs according to sex is to be seen.

The mechanism by which the anabolizing androgen hormones caused an enhanced activity in hepatic microsomal enzymes is discussed.

154 The Influence of Drugs given 1—72 Hours Previously upon Hexobarbital-Narcosis and upon Bemegride-induced Convulsions in Mice. C. L. Rümke (Holland).

Several drugs prolong the duration of narcosis when given 1 hour before the administration of hexobarbital. In mice it has been observed that (a.o.) chlorpromazine, promazine, hydroxyzine, orphenadrine, phenobarbital, glutethimide, urethane, trimethadione, Mesontoin, primidone, iproniazid and SKF 525A decrease the duration of hexobarbital narcosis if a single dose of one of these drugs is given 24–72 hr previously. For some of these drugs a correlation between this effect and a markedly increased activity of drug-metabolizing enzymes in the liver microsomes has been observed by various authors.

In mice the convulsant effect of intravenously injected bemegride was measured by determining the dose which causes convulsions in 50 per cent of the animals (CED_{50}). After chlorpromazine, hydroxyzine, orphenadrine, SKF 525A, Panthesine, serotonin, pipradrol and azacyclonol the CED_{50}'s were found to be decreased, and after phenobarbital, glutethimide, trimethadione, Mesontoin, primidone, phenacemide, atropine, morphine and urethane they were found to be increased, if the pretreatment had been given 1 hr previously. After a single dose of SKF 525A, administered 48 hr previously, the CED_{50} of bemegride was found to be larger than that in saline pretreated controls. Single doses of phenobarbital, glutethimide, Mesontoin

and primidone, given 24–48 hr earlier, resulted in a decreased CED_{50}.

The enhancement of the convulsant effect by the "anticonvulsant drugs", given two days previously and the decreasingly effect upon the duration of the hexobarbital-narcosis must depend of different modes of action. Twenty-four hr after phenobarbital administration the narcosis is shortened already, whereas there is still a marked anticonvulsant action.

155a Rolle des R.E.S. im Abbau einiger Barbiturate. M. Timar, E. Thomas, S. Sauvard und E. Repciuc (Rumänien).

Der Beweis für eine bedeutende Rolle des R.E.S. im Abbau des Evipans und des Amitals wurde auf folgenden Wegen geführt:
(1) Subtotale Resektion der Leber der Ratte, wobei eine Verlängerung der Barbituratnarkosedauer festgestellt werden konnte.
(2) Blockade des R.E.S. durch Makromoleküle (Methylcellulose, Intradex, Macrodex, etc.), wobei gleichfalls eine Verlängerung der Narkosedauer festgestellt werden konnte.
(3) Totale sublethale Bestrahlung der Versuchstiere vertieft die Wirkung der vorausgehenden Eingriffe.
(4) Trypanblau ist wirkungslos; Bestrahlung vor Trypanblauverabreichung ist gleichfalls wirkungslos. Bestrahlung nach Trypanblauverabreichung ist hingegen sehr wirksam.
(5) Es besteht somit ein grundlegender Unterschied in der Wirkung zwischen Makromolekülen und dem Trypanblau.

155b Role of Reticulo-Endothelial System in the Metabolism of Some Barbiturates. M. Timar, E. Thomas, S. Sauvard and E. Repciuc (Roumania).

Evidence of a significant role of the reticulo-endothelial system in the metabolism of Evipan and amytal was obtained as follows:
(1) Subtotal resection of the rat liver, whereby lengthening of the duration of barbiturate narcosis was established.
(2) Blockade of the reticulo-endothelial system by macromolecules (methylcellulose, Intradex, Macrodex, etc.) which also resulted in a longer period of narcosis.
(3) Whole-body sublethal irradiation of the experimental animals heightened the influence of the preceding interventions.
(4) Trypan blue was ineffective—irradiation before administration of trypan blue was also ineffective although irradiation after administration of trypan blue was very effective.
(5) There is thus a fundamental difference between the action of macromolecules and trypan blue.

156 Direct Comparison of ^{14}C-guanethidine (Ismelin) Excretion following Oral and Subcutaneous Administration. B. Calesnick, H. Sheppard, N. Bowen and J. Kamdar (U.S.A.).

Guanethidine, labelled with ^{14}C and having a specific activity of 15 μc/mg, was administered to an adult female patient. Fourteen days after a single oral dose of 50 mg, she received a single subcutaneous injection of 10 mg. Following each route of administration, total bile and urine were obtained hourly for the first 6 hr and pooled during the 6–12 and 12–24 hr periods. Bile was collected through a biliary fistula.

After oral adminstration, a maximum urinary excretion rate of 22·1 μg/ml/hr developed between 5–6 hr and the total 24-hr urinary recovery was 5·28 per cent. Two maximum biliary excretion rates developed between 2–3 and 4–5 hr and the 24-hr biliary recovery was $1{\cdot}4 \times 10^{-2}$ per cent.

After subcutaneous administration, a maximum urinary excretion rate of 25·9 μg/ml/hr developed between 0–1 hr and the total 24-hr biliary recovery was $9{\cdot}4 \times 10^{-2}$ per cent.

Similar double-maximum biliary excretion rates have been observed with studies of [^{3}H]hydrochlorothiazide and suggest either dual sites of intestinal absorption or a hepatic mechanism for temporary storage and release of these compounds.

These data also suggest that guanethidine is slowly absorbed and excreted since traces of this drug were found in the urine and bile 14 days after oral administration. In addition, the 24-hr urinary excretion is increased approximately 5-fold when the subcutaneous route is used.

157 Introducing the "Irradiation Cycle Rate" in Microwave Radiation Exposures. W. B. Deichmann (U.S.A.).

Microwaves are beginning to gain considerable recognition as a scientific and biological tool. Like light, microwaves may be directed, focused, reflected, refracted or concentrated. In biological material, they may be reflected or absorbed or they may penetrate. This may be associated with, or without, the production of heat, resulting in: changes in the structure of proteins and carbohydrates; behaviour changes; changes in the response to established reflexes, etc.

Out studies (24,000 Kmc) have focused attention on certain factors, which, unless recognized and controlled, will influence the effects of a specific dose of irradiation. These include time, air currents, temperature and body wt.

In 1958, we rendered a preliminary report on another factor which also must be given utmost consideration to obtain reproducible results. It was found that in an intermittent or interrupted cyclical exposure, the effects of a specific dose of irradiation, for instance, exposure to 100 mw/cm^2 for 30 sec/min, will depend on the length of time, of

the individual bursts of exposure, i.e. 1 sec "on"—1 sec "off", 5 sec "on"—5 sec "off", 10 sec "on"—10 sec "off", and similar patterns. It was found that the briefer these are, the less are the local and systemic effects in animals, even though the total dose of irradiation was constant per unit of time (in our experiments, 1 min).

To recognize this phenomenon, we are introducing the term "irradiation cycle rate", which is defined as "the number, or the rate at which on—off periods of irradiation are repeated per unit of time". The significance is obvious when considering rapidly- and slowly-turning radars.

(The investigation was sponsored by the Rome Air Development Centre Air Research and Development Command, United States Air Force.)

158 Electroshock Seizure Responses to Psychopharmacological Agents after 500 r Whole-body X-irradiation in Rats. F. ROSENTHAL and P. S. TIMIRAS (U.S.A.).

The present authors and others have demonstrated that whole-body X-irradiation alters brain function. This study investigates the effects of a sing,e moderate, whole-body X-ray exposure (500 r) on the electroshock convulsive response to reserpine (2 mg/kg), chlorpromazine (5 mg/kg), iproniazid (100 mg/kg) and dilantin (40 mg/kg). Eighty-four adult female rats were grouped as follows: controls, irradiated controls, 4 groups of non-irradiated and 4 groups of irradiated rats injected s.c. with the respective drugs. Electroshock seizure threshold (EST) was measured from day 1 to 20 post-irradiation. The pattern of maximal electroshock seizures (MES) and duration of postictal depression (PID) were determined 5 and 17 days post-irradiation. The main results were: (1) irradiation increased brain excitability and slightly prolonged postictal depression; (2) by the fifth post-irradiation day, reserpine significantly lowered EST in control and irradiated rats. Chlorpromazine, iproniazid and dilantin did not modify EST in controls. After X-ray exposure, chlorpromazine and iproniazid enhanced the increase in excitability produced by irradiation whereas dilantin counteracted this excitatory effect by raising EST significantly above control values; (3) reserpine markedly prolonged the duration of total MES, tonic extension and clonus while shortening tonic flexion. Chlorpromazine and iproniazid did not significantly modify MES with or without irradiation. The anticonvulsant action of dilantin (shortening of total MES and tonic extension, prolongation of tonic flexion) was more pronounced in irradiated than in control rats; and (4) PID was prolonged by chlorpromazine, reserpine and iproniazid and this effect was more marked after irradiation. Thus, of the 4 drugs studied, chlorpromazine, iproniazid and dilantin had an intensified convulsant and anticonvulsant effect after irradiation.

159 Mode of Action of a Number of Radio-Protective Compounds. C. VAN DER MEER (Holland).

The oxygen tension in the spleen and bone marrow of unanaesthetized mice and rats was determined with a polarographic technique. With this and other techniques, used under conditions which prevail during irradiation, it was found that the radio-protective compounds studied can be divided in two groups.

Group I. Compounds belonging to this group protect by causing hypoxia of the blood-forming organs. A correlation exists between the percentage reduction of the oxygen tension in the spleen caused by either the injection of these compounds or by lowering the oxygen content of the inspiratory air and the percentage protection against radiation. This group comprises: (a) "Biologically active" amines: histamine, epinephrine, tryptamine, 5-hydroxy-tryptamine. Norepinephrine does not protect our mice and does not cause a significant hypoxia in the spleen; (b) Cyanide, KCN in protective doses causes severe vasoconstriction due to vasomotor stimulation; and (c) Sodium-diethyl-dithiocarbamate.

Group II. These compounds do not cause hypoxia of the blood-forming organs. Their mode of action is not established. This group comprises the "SH-compounds" cysteine, cysteamine, AET.

The hypoxia in the spleen caused by cystamine is insufficient to fully explain the protective activity. Possibly cystamine, in itself an amine but convertible into an "SH-compound" in the body, combines the mechanisms of groups I and II.

160 Role of the Central Nervous System in Processes of Restitution and Compensation under the Effect Radio-protective Agents. S. V. ARBUZOV (U.S.S.R.).

Progressive extension of the peaceful uses of nuclear power has aroused growing interest in research on chemical, or on what has rightly been called pharmacological protection. Today this rapidly expanding subject has actually become a special branch of pharmacology—radiation pharmacology. Studies by the author and his collaborators involved search for new agents and investigation into certain features, peculiar to the mechanisms underlying their radiation protective or therapeutic effects. The following facts have been established:

(1) Mercamine is considered to belong to the drugs which tend to be distributed uniformly in organs and tissues. Quantitative differences have been rebealed in the rates of its accumulation. In the central nervous system, 3 hr after the administration of mercamine-^{35}S, the amount of activity concentrated within the cerebral cortex proved to be higher than that in other organs. At 6 hr, activity of the mercamine-^{35}S had decreased, while it was found to have risen in subcortical regions. Twenty-four hours after the administration of labelled

mercamine, almost all of the cerebral tissue activity proved to be concentration within the brain stem.
(2) Mercamine depresses the process of excitation in the cerebral cortex. At segmental level, the central nervous system is not so sensitive to the effects of this compound. A certain role in the display of defence responses evoked by mercamine administration is shown to belong to the sympathetic nervous system.
(3) Preliminary administration of the sulphur-containing radio-protective compounds brings about a sedative effect, evolving with time. The physiological significance of the preliminary response to administration of these protective drugs, displayed by the animal body as a whole, depends on primary prevention of and compensation for injury underlying the eventual marked depression of potential rhythms affecting the cortex, hypothalamus sympathetic nervous system, etc. Accordingly, total changes in bio-potentials, presented as pathological rhythm depression, proved to be greater in unprotected as compared to protected animals.
(4) Imidazole 2-carbonic acid compounds related to caffeine owing to their sedative effective, prevent any pathological alterations in central nervous system reactivity, excitation or depression alike. Such a selective effect, exhibited by these compounds, favours prevention by compensating for radiation injury.
(5) Preliminary administration of neuroplegic agents has been shown to reduce the extent of impairment and to shorten the time of recovery of a number of unconditioned responses in treated irradiated animals, as compared to untreated controls. This may be attributed to restitution of functions related to subcortical controlling mechanisms.

It has been shown that the use of various neuroplegic and autonomic drugs, as well as of chemical agents affecting mediation of nerve impulses, for their therapeutic or preventive effects against radiation injury should be considered as a promising line of attack.

161 Effects of Radiomimetric Alkylating Agents on Lymphoma Cells in Tissue Culture. P. Alexander and B. Mikulski (United Kingdom).

Nitrogen mustards at doses at which they killed lymphoma cells in tissue culture produce a significant amount of cross-linking of the DNA within the nucleus and this reaction seemed adequate to explain the growth-inhibiting effect of these substances on dividing cells. While it was possible to achieve some cross-linking with myleran, the dose that had to be used was so great as to make it unlikely that this reaction was responsible for its cell killing activity. Further evidence for a difference in action between these two classes of alkylating agents has been observed by studying the growth characteristics of mouse lymphoma cells in tissue culture. HN2 immediately arrests cell division while after dimethyl myleran (and X-rays), at least one division occurs before cell proliferation stops. After a time, depending on the dose, the cell number goes up again, in cultures treated either with the chemicals or with radiation. This is due to the cells which survive the initial treatment and which grow up in the culture. By extrapolation, the number of these "survivors" can be computed on the assumption that the doses used did not introduce a significant mitotic delay. This seems reasonable, since with X-rays even the affected cells (i.e. those that eventually degenerated) divided at the normal rate immediately after treatment.

The cessation of cell division (immediate for HN2 and delayed with dimethyl myleran) is not due to the inhibition of DNA synthesis which proceeds normally for a day after there has been no increase in cell numbers. After all the treatments, the cells increase in size; with HN2 the response is quite uniform and the cell volume is doubled, after which there is degeneration. With dimethyl myleran and X-rays a wide spectrum of cell sizes is obtained and a small proportion become giants with volumes more than ten times those of the control. The microscopic appearance of the cells varies; after dimethyl myleran and X-rays the increase in size is the only observable change before degeneration, while the HN2 treatment produces distinct granularity after 24 hr.

As far as cytoxic action is concerned dimethyl myleran appears to be truly radiomimetic, while HN2 would appear to kill cells by a mechanism that is quite different from X-rays. Sensitization by exposing the cells to 10^{-4} iodoacetate for 30 min follows the same pattern; it increases the cytotoxic action of X-rays and of dimethyl myleran but does not influence the effects produced by HN2.

162 Effect of Various Substances on X-ray Irradiation of Bilirubin. G. Barac, M. L. Beaumariage, C. Cuvelier and W. Notay (Belgium).

Radiation damage *in vivo* is thought to be due to the production of free radicals which are potent oxidizing agents. Chemical protection against irradiation should therefore be afforded by substances such as cysteamine which antagonize the effect of these radicals. The study of both radiation damage and chemical protection *in vivo* requires also careful investigations of these processes in simple chemical systems of biological value *in vitro*.

Bilirubin is spontaneously oxidized in an aerated aqueous solution. The rate of this oxidation is greatly enhanced by X-rays. The oxidation of bilirubin in chloroform or acetone is similarly accelerated by X-rays. Pure bilirubin is not directly oxidized by X-rays. The oxidation of bilirubin in aqueous solution by X-rays is reduced or abolished by various anti-oxigenic substances such as ascorbic acid, cysteine, cysteamine, 5-hydroxytryptamine, etc., or by serum albumin

which binds bilirubin. Ovalbumin and gamma-globulin which do not bind bilirubin do not affect the action of X-rays. Alpha-tocopherol strong inhibits the action of X-rays or bilirubin.

These results and several others will be compared with those obtained in the study of other systems *in vitro* and also with those already known *in vivo*.

163 Change in the Effect of Thiopental Sodium in Irradiated Rats and Its Causes. V. Grossmann (Czechoslovakia).

The reactions of an organism are changed by irradiation and these changes develop with the development of irradiation disease. In such case, changes in the effect of drugs may, broadly speaking, be conditioned by changes in metabolism or change in sensitivity of the target organ. We have observed the changes in the effect of thiopental sodium on the third and sixth day after irradiation in rats irradiated with a dose of 600 r. Thiopental sodium was determined in the urine, brain tissue and blood by Brodie's technique.

By this means, it was found that a statistically insignificant fall in the blood level of thiopental sodium, a slight increase in its excretion in the urine and a prolongation of its presence in the brain occurred in irradiated animals. The duration of the effect, measured by the time elapsing until the first change of position of the body, is also prolonged both on the third and sixth day after irradiation. In control animals, the time taken for thiopental sodium to disappear from the brain and the time of arousal are the same, but this is not the case in irradiated animals. The time of arousal is prolonged in comparison with the time taken for thiopental sodium to disappear from the brain, this prolongation being statistically significant on the sixth day.

The above results suggest that the prolongation of the inhibition after the administration of thiopental sodium in irradiated animals is conditioned by two factors. One is the prolonged presence of thiopental sodium in the brain, although it cannot be excluded that some of it consists of thiopental acid. The second factor is the decreased ability of the central nervous system to recover from the inhibition following thiopental sodium to normal. This factor is especially marked on the sixth day after irradiation.

164 Redox Processes in Liver Homogenates of Irradiated Rats. J. Kvetina and V. Grossmann (Czechoslovakia).

The ability of liver tissue homogenates to decolourize methyleneblue as an indicator of redox potency was investigated in homogenates of irradiated animals in comparison with controls.

In the first phase the ability of the homogenate to decolourize methylene blue is decreased. This seems to be a consequence of metabolic changes in the whole animal after irradiation as direct irradiation of homogenates is without effects. To differentiate substrate and enzyme damages, experiments with mitochondrial fractions of irradiated animals were performed. The addition of succinate does not prevent the reduction of the potency to decolourize methylene blue.

The same results are also obtained in whole liver homogenates, when succinate is added, although at least some effect was observed. The results are discussed.

165 The Pharmacology of some substituted Aryloxyalkanoic Acids. S. S. Adams (United Kingdom).

A number of substituted aryloxyalkanoic acids have been investigated for their activity in delaying ultra violet erythema in guinea pigs. An interesting structure activity relationship has been established and two of the more active compounds were subjected to a more extensive pharmacological investigation.

The most active member of this series was found to be clinically inactive in rheumatoid arthritis. These findings must cast doubt on the suggestion of Winder[1], and Adams and Cobb[2] that anti-erythemic activity might be directly related to clinical anti-rheumatic activity, and will be further discussed.

In the course of toxicological studies it was found that one of the members of this group was a potent ulcerogenic agent in dogs. The nature and type of this damage will be discussed and illustrated.

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1. Winder, C. V., Wax, J., Burr, V., Been, M. and Rosiere, C. E. (1958), *Arch. int. Pharmacodyn*, **116,** 261.
2. Adams, S. S. and Cobb, R. (1958), *Nature (Lond.)*, **181,** 773.

Wednesday. August 23

166 Passage of Drugs Across the Gastro-intestinal Epithelium. L. S. Schanker (U.S.A.).

Absorption studies with the stomach, small intestine, and colon have revealed that the gastro-intestinal epithelium behaves as a lipid-like barrier to the passage of many drugs and other foreign organic compounds. Drugs administered in true solution are readily absorbed in their lipid-soluble, undissociated form, and very slowly absorbed in their lipid-insoluble, ionized form. Moreover, with drugs that are mainly undissociated, the rate of absorption is roughly related to the lipid/water partition coefficient of the undissociated form—the greater the coefficient, the more rapid the rate of absorption. In the stomach, where the contents are strongly acid, acidic drugs like the salicylates and barbiturates exist as undissociated molecules and are readily absorbed; in contrast basic drugs, including many plant alkaloids, exist largely as ions and are very slowly absorbed. In the small intestine and colon, where the contents have a pH of 6 to 8, most weak acids and bases are at least partially undissociated and are absorbed at various rates depending on their lipid-solubility. Slowest rates of intestinal absorption are observed with completely ionized drugs like quaternary ammonium compounds and sulphonic acids, and with lipid-insoluble molecules like sulphaguanidine and mannitol.

Although the gastro-intestinal absorption of most foreign organic compounds may be explained in terms of simple diffusion across a lipid-like boundary, there is evidence that a drug may also be absorbed by a specialized transport process if its chemical structure is similar enough to that of the natural substrate. For example, the foreign pyrimidines, 5-fluorouracil and 5-bromouracil, are actively transported across the intestinal epithelium by the process which transports the natural pyrimidines, uracil and thymine.

167 Biliary Excretion and Choleresis. I. Sperber (Sweden).

The transport mechanism handling bile salt in the liver appears closely similar to the renal tubular mechanism secreting, e.g. phenol red and hippuric acid. This mechanism may transfer into the bile (in addition to bile salt) a great number of other substances, mostly aromatic acids of a molecular wt. of 350–700. Several of these compounds are excreted in comparatively large amounts, and have a choleretic effect. To obtained information concerning the nature of this choleretic effect the concentration of such substances in bile has been examined. To reduce, if possible, the influence of variations in bicarbonate secretion and reabsorption, and the basal excretion of bile salt, only those experiments are included, where the bile flow has been more than doubled by the choleretic effect of the substance examined.

In experiments with chicken, cholate, taurocholate, dehydrocholate, phlorizin, phenol red and polyethylene glycol have been used. Further experiments have been made in the turkey, pigeon, goat, rat, rabbit and guinea pig. The concentration of these substances is usually between 25 and 60 mM/a., except for the micelle-forming cholate and taurocholate, which show decidedly higher concentrations, and polyethylene glycol (usually lower concentration).

The results obtained are considered to be in satisfactory agreement with the hypothesis that the secretion of bile salt (and similarly handled compounds) into the bile capillaries is the primary event in bile formation. The osmotic effect of these large ions may be supposed to result in the inflow of water and dissolved small ions and molecules.

168 Renal Excretion of Weak Organic Acids and Bases. G. H. Mudge (U.S.A.).

The renal excretion of certain organic acids and bases is determined by filtration, active secretion and passive back diffusion. Secretion involves a minimum of two separate mechanisms, one for acids and one for bases. With the increasing number of compounds which are being demonstrated to be actively transported, specific structural requirements for transport remain difficult to define. Competitive inhibition of apparent secretion is the most reliable criteria for identifying an active transport component. The degree of tubular reabsorption varies greatly, from (for example) *p*-aminohippurate, which is virtually non-reabsorbed, to probenecid, which is normally completely reabsorbed. Within series of analogues the rate of urinary excretion is a function of four independent variables. Acid strength (p*Ka*) and lipid solubility are inherent chemical characteristics; urine pH and volume are biological variables. There is no convincing evidence that any of these factors determines the rate of active secretion. The experiments are consistent with the hypothesis that each of these factors influences the rate of non-ionic back diffusion through a lipid membrane, presumably the distal tubule, but not necessarily excluding the proximal tubule. The concept of continuous and simultaneous active secretion and passive reabsorption imposes rigid experimental restrictions on the study of active transport by either the clearance or tissue slice techniques. Most products of drug metabolism are less lipid soluble than the parent compound and, hence, appear to be eliminated by the kidney as a result of decreased reabsorption.

169 Effect of Drugs on Renal Transport Mechanisms. R. W. Berliner (U.S.A.).

The wide variety of transport processes in the kidney and the fact that they may be modified by a number of drugs and hormones makes it necessary

to limit the scope of the material to be covered in this presentation. Consideration will be limited, therefore, to those agents which have major influences on the transport and excretion of strong monovalent electrolytes. Although these transport processes are subject to modification by the effects of many drugs, and these effects are frequently utilized for therapeutic purposes, information concerning the mechanisms by which these effects are produced is, at best, fragmentary. The effects of several agents are of particular interest because they relate these transport processes to others in systems which are more easily subjected to direct study. Among these are: (1) the agents derived from or related to the glycosides of digitalis; (2) inhibitors of carbonic anhydrase; and (3) pituitary antidiuretic hormone. Two other groups, the mercurial diuretics and the benzothiazide derivatives, are of particular importance because of the intensity and therapeutic usefulness of their effects but, unfortunately, virtually nothing is known of their mechanism of action and even the locus of this action is in dispute. Discussion will be concerned with what may be inferred about the nature of renal tubule electrolyte transport from the effects of these drugs.

170 Discussion of the Previous Paper. F. Berglund (Sweden).

171 New Aspects of Cardiac Glycosides. Introductory Remarks. W. Wilbrandt (Switzerland).

172 Stereochemistry of Glycosides as Related to Biological Activity. C. Tamm (Switzerland).

The aglycone moiety is responsible for the physiological effect of the cardiac glycosides. Therefore, the stereochemistry of the cardenolides and the bufadienolides (toad poisons) is discussed with emphasis on the conformational aspects. The cardiac aglycones differ in their structure from the steroids of other biological activity in a characteristic manner.

On the basis of the geometric mean of the cat lethal doses (LD) a detailed analysis of the activities of the various structural types of glycosides is presented: (1) Effect of the sugar unit if combined with the same aglycone; (2) Effect of the aglycone unit if combined with the same sugar; (3) Influence of the number of sugars; (4) Effect of the nature of the unsaturated lactone group; (5) The role of the position and the stereochemistry of the functional groups in the aglycone; (6) The importance of the nature of the glycosidic linkage in the glycosides; (7) Activity of chemical derivatives of aglycones and glycosides.—Unpublished results are included in this consideration.

Finally, the scope and limitation of the assay method used above and the relationship to other biological methods are discussed. Furthermore, the possibilities of employing such results for the evaluation of therapeutical use are considered.

173 Possibilities of Further Development in the Glycoside Field by Modifying the Glycoside Structure. K. K. Chen (U.S.A.).

More than 30 years ago we started to investigate the chemistry and pharmacology of Chinese toad poison.[1] Our interest was focused on digitalis-like substances. This was followed by isolation of similar products from 14 species of toads, all of *Bufo* genus. Our work was soon extended to glycosides and aglycones of plant origin. To date, cat-assay results and other observations on about 300 compounds, mostly supplied by Reichstein and Stoll, have been accumulated suitable for consideration of structure–activity relationship. As a rule, cardenolides are weaker than bufadienolides of the same configuration. The glycosides of cardenolides are generally more active than their parent aglycones, but those of bufadienolides frequently have a potency equal to or lower than their aglycones. Among the cardenolides, acetylation of the OH-group at C_3 or C_{16} has a favourable influence on cardiotonic activity. The aldehydes at the angular C_{10} are stronger than the methyl analogues. Epimerization at C_3, α-orientation at C_5 or saturation in the lactone ring results in a decrease or disappearance of action. Thirty-eight compounds were tested in patients with ventricular fibrillation, but they are all less easily absorbed through the gastrointestinal tract than digitoxin. The renotropic effect, speed of action, absorbability, and emetic action of glycosides will be discussed.

1. Chen, K. K. (1929), *Proc. Soc. Exper. Biol., N.Y.*, **26**, 378.

174 Metabolism of Cardiac Glycosides. K. Repke (Germany).

This review considers the metabolic fate of cardenolides but only in so far as facts may be related to their mode of action.

The double bond in the lactone ring may be reduced, but this reaction proceeds slowly and irreversibly so that this part of the molecule cannot serve as a hydrogen carrier in intermediary metabolism. Hydroxylation at C_{12} is not a necessary prerequisite for activity. The glycosides may undergo fission in the body to produce the corresponding genins as intermediary products. The aglycones may function as coenzymes of a transhydrogenase, but it has been shown that this effect has no relation to their mode of action. The glycosides are acting directly as such. The importance of the sugar component consists in favouring enrichment of the molecules in the heart and in preventing rapid inactivation by epimerization and conjugation at C_3-OH.

Digitoxin is not more enriched in the heart and, in part, even less than in other organs. The subcellular distribution has not been clarified beyond doubt as yet. The overall distribution will not

differ greatly in the various animal species. The velocity and direction of enzymatic transformations of glycosides present great variations in different species, though all these variations do not cause the differences in their susceptibilities to glycosides. Some observations lend support to the idea that cardiac poisons act in influencing enzymatic systems. The possibility cannot be excluded that, in the various organs or animal species, such enzymes might be distributed unequally or possess different glycoside sensitivities.

175 Cardiac Glycosides and Heart Metabolism. R. J. Bing (U.S.A.).

The discussion deals with the action of digitalis on the energy production and energy utilization of the heart. Cardiac glycosides produce no change in myocardial utilization of substrates. However, myocardial release of potassium has been reported. Using ^{14}C-labelled digitalis, increased utilization of glucose by *in vitro* preparations of heart muscle was demonstrated. No effect of cardiac glycosides on ATP– induced contractility of actomyosin bands and glycerinated heart muscle strips could be demonstrated. The action of digitalis on the membrane function is demonstrated by shortening of membrane action potential. Apparently, in contrast to catechol amines acting on energy production (phosphorylase), digitalis exerts its effect primarily by changing the intracellular ionic composition. Increased contractility of actomyosin is the result.

176 Autoradiographic Localization in the Tissues of Drugs and Metabolites. S. Ullberg (Sweden).

Methodological improvements in autoradiography with water-soluble substances and in labelling techniques are factors that have rapidly increased the significance of autoradiography as a pharmacological research method.

Compared with histochemistry, autoradiography gives slightly less good resolution, but, on the other hand, is generally more suited for following a time sequence and gives the opportunity to change between distribution surveys and detailed pictures.

Tissue localization of an injected drug generally changes rapidly with time. A good way to start a distribution and metabolism study may therefore be to make a whole animal autoradiographical survey on a number of small animals killed at different times after a single intravenous dose.

Then microautoradiographical localization in selected tissues and chemical identification of metabolites may be planned with guidance of the whole animal autoradiographs.

Examples of problems which are well suited to be attacked with autoradiography are localization in effector organs, the accumulation of presumed precursors in biosynthetic loci, and limitation of distribution by physiological and pathological barriers.

A number of methodological problems and possibilities will be discussed and illustrated with different applications.

Among the methological aspects that will be considered are quantitative autoradiography, choice of isotope and its influence on the autoradiographical resolution, the possibility of using short-lived isotopes, the possibilities of using and the advantages of double tracer autoradiography, and selective chemical extraction from the histological section before autoradiographical exposure.

177 On the Action of Some Pharmacological Factors upon Nerve-free Cells (Infusoria) and upon the Cells of Stretch Receptors in Arthropods. C. S. Koshtoyants, N. N. Kokina and B. Tashmukhamedov (U.S.S.R.).

The authors noticed that under the influence of acetylcholine and anticholinesterase substances, the motor reaction characteristic of Paramecia in a galvanic field changes sharply: negative galvanotrophism either disappears or is replaced by a positive one.

By introducing glass micro-electrodes into motile cells of parasitic Infusorium, *Opalina ranarum* and by oscillographical registration of periodical bioelectrical activity of these animals, the authors studied the action of acetylcholine, anticholinesterase substances, β-alanine and γ-aminobutyric acid (GABA) on this activity. The oscillograms obtained clearly show that under the influence of anticholinesterase substances the rhythmical bioelectrical activity of the nerve-free unicellular organisms studied undergoes successive phases of excitation and inhibition, while under the action of β-alanine and GABA a depression of rhythmical electrical activity of a different duration in the case of each of these substances takes place. These experiments illustrate the cellular level of action of typical neuropharmacological agents.

Comparative pharmacological observations on the cells of stretch receptors in Crustacea (*Astacus*) and insects (*Bombyx*, *Antheraea*) showed that GABA which is known to depress the activity of stretch receptor cells in Crustaceans does not inhibit the activity of the same receptors in insects. Oscillograms obtained when studying GABA effect upon stretch receptors in the insects mentioned at the stage of larva, pupa and moth will be presented. In searching for agents inhibiting the activity of stretch receptors in insects we found an inhibiting effect with tricresol. The results show different sensitivity to pharmacological agents in the cells of the same functional importance (stretch receptors) but belonging to phylogenetically different groups.

178 Use of Microgasometric Techniques in Pharmacological Studies. E. Giacobini (Sweden).

Conventional histochemical techniques based on the staining of tissue sections are generally limited by certain sources of error such as: staining unspecificity, difficulty in locating the staining pro-

duct and distribution or partial inactivation, due to the fixation and embedding procedure. Moreover, they are mostly unsatisfactory for quantitative comparison purposes. For this reason micro-quantitative techniques seem to be better adapted to fine metabolic studies at tissue or cellular level. These techniques, however, involve a certain degree of technical difficulty. A relatively simple gasometric microtechnique for analysis at cellular level is the cartesian diver of Linderstrom–Lang which has been employed during the past 20 years for different biological studies. By means of this technique it has recently been possible to start a series of neuropharmacological investigations at the cellular level and to study in particular the following: (a) the localization of several types of enzymes in different nerve cells belonging to the same nucleus or structure (e.g. cholinesterase in anterior horn cells of the spinal cord), or in nerve and glial cells of the same nucleus (e.g. carbonic-anhydrase in central nuclei), (b) the distribution of different types of enzymes in the same unit of physiological or pharmacological importance (e.g. through a whole reflex arch), (c) the intracellular localization of one or several enzymes, e.g. the acetylcholinesterase in the anterior horn cell of the spinal cord and (d) to perform a polyenzymatic analysis in a single neurone. The following enzymes have been hitherto determined in the same neurone such as: cholinesterase (acetyl- and buthyril-), oxidase (cytochrome- and succino-), anhydrases (carbonic-anhydrase).

The results of this investigation may be of value in the study of the cellular pharmacology and be integrated with those obtained by other techniques applied at cellular and subcellular level.

179 Potentialities of Quantitative Histochemistry for the Study of Nervous System Pharmacology. O. H. Lowry (U.S.A.).

Drugs which act on the central nervous system in a useful manner have differential actions on the various functional systems present. The extreme complexity of the brain makes it difficult to study the chemical bases for these differential effects. Quantitative histochemical techniques are available which make it possible to study isolated samples of brain of the size of a small nerve cell body (10^{-4} microgram). The brain is quick-frozen, sectioned in a cryostat and dried at −40°C. Without fixation or staining, samples are dissected for weighing and analysis. Simple balances permit rapid weight determination to 1 per cent. Older methods permit the measurement of a wide variety of enzyme activities in such material. Such measurements have some relevancy for pharmacology in that they indicate the metabolic capacities of the various components of the nervous system. More recently, analytical sensitivity has been increased almost without limit, which makes it possible to study the levels of substrates and neuroeffectors even if present at very low concentrations. This should permit the study at the cellular level of the chemical effects of drugs on the central nervous system.

180 Recent Developments of Micromethods for the Study of Renal Physiology and Pharmacology. K. J. Ullrich (Germany).

To obtain information about tubular transport systems and pharmacological effects thereon, it is often necessary to perform micropuncture studies on individual parts of the nephron.

As technical progress in this field in the past few years the following will be considered: (1) A more effective immobilization of the kidney; (2) withdrawal of fluid through glass capillaries with ground tips and polyethylene catheters; and (3) new analytical methods—microcryoscopy, microelectrotitration, flame photometry—and construction of suitable photometric devices. These first three categories have been described in publications from this and other laboratories, so we shall elaborate only on (4). To measure the transtubular flux of NaCl and non-electrolytes, a method of "stop-flow-reabsorption" has been elaborated by Gertz and Ullrich. Because the proximal tubular fluid and late distal tubular fluid in antidiuresis are isotonic, a net transfer of solutes must be linked with a corresponding water transfer. The tubule is blocked by injection of coloured oil. Then a small amount of test fluid is injected into the oil dividing the oil column. The volume change of the injected fluid is photographed. Isotonic saline is reabsorbed from the proximal tubule of normal rats with the half volume time of 10 sec. The corresponding NaCl flux is $8{\cdot}20 \times 10^{-4}$ μM Na/mm²/sec (Gertz) and the transtubular potential 20 mV (Stöckle). After intratubular injection of isotonic maltose or mannitol solution first an inward flow of NaCl and water is observed then a slow reabsorption. During reabsorption a steady state NaCl and non-electrolyte concentration is achieved and the ratio Na efflux/non-electrolyte efflux must be proportional to the intratubular Na concentration/non-electrolyte concentration. If the latter is known the individual fluxes can be calculated. If NaCl and non-electrolyte are present in the tubule, the Na efflux is much smaller than with isotonic NaCl alone.

Finally, if a nonpermeable nonelectrolyte is injected into the tubular lumen, there is no net flux of any solute. In this case the electrochemical gradient of the ions can be determined by measuring electrical potential and concentrations on both sides of the tubular membrane. Then the electrochemical gradient indicates (1) whether an ion species is actively or passively transported, and (2) how large the active force for the actively transported ion is.

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181 The Binding of Inhibitors to Carbonic Anhydrase in vivo: Drugs as Markers for Enzyme. T. H. MAREN (U.S.A.).

Acetazolamide and congeners bind to carbonic anhydrase containing tissues *in vitro* in a manner quantitatively predictable from the inhibition constant of drug (K_i) and the molar concentration of enzyme (E_0) in the tissue preparation.[1] In the present study, binding *in vivo* to red cells, kidney, and choroid plexus was measured (I_{bound} or EI) and compared to (E_0) in untreated animals. Five mg/kg of acetazolamide was injected i.v. into dogs whose tissues were analyzed for drug at different times within the following week. The rate constant for initial (first day) plasma decay is 0·4 hr^{-1}; on this basis only, plasma concentration would be 10^{-3} μg/L at 48 hr. Measured plasma concentration, however, was approximately 30μg/L; this agrees with the theoretical value for free drug (I_f) in equilibrium with drug (about 5 mg/L) found bound to carbonic anhydrase in red cells (EI), according to the expression $I_f = \frac{(EI) \times K_i}{(E_0) - (EI)}$.

After the first day, the steady state between (EI) in red cells, (I_f) in plasma, and urinary (I_f) is such that drug decay is very slow; the rate constant is about 0·3 $days^{-1}$, and equilibrium between (I_f) in plasma and (EI) in other tissues is fairly well maintained. The latter was measured in kidney and choroid plexus, again by analysis for inhibitor. These measurements agreed well with independent enzymic estimates of (E_0) in those tissues. It is suggested that under these conditions the recognition of inhibitor in organs represents specific combination with carbonic anhydrase, and that intimate cytological localization of inhibitor by appropriate labelling may yield information regarding the enzyme.

1. MAREN, T. H. *et al.* (1961), *Biochem. Pharmacol.*, **6**, 21.

182 Direct Extracellular Application of Drugs. D. R. CURTIS (Australia).

Methods will be discussed whereby pharmacological agents can be applied electrophoretically into the extracellular environment of mammalian neurones. The use of multibarrel electrodes enables the electrical responses of the cells to be observed and interactions between different compounds upon a single unit can be studied. The combination of intracellular recording and extracellular drug application permits the mode of action of the drug to be determined. Difficulties inherent in the localization of the site of drug action following intravenous, intra-arterial or intrathecal administration are obviated. The technique is not without disadvantages, the major one being the difficulty in estimating the drug concentration attained.

183 Relationship between Structure and Biological Activity (Continued). Introductory Remarks. A. H. THEORELL (Sweden).

184 Biological Specificity in Protein Small Molecule Interactions. D. E. KOSHLAND, JR. (U.S.A.).

The ability of proteins to distinguish between closely similar structures is of fundamental importance in such diverse biological phenomena as drug–receptor, hapten–antibody, substrate–enzyme and inducer–induced enzyme systems. Although the detailed understanding of this specificity is unknown in any case, the specificity patterns are sufficiently similar to each other to suggest that a mechanism with many common features operates in all of these systems. Some of the features of this mechanism are: (a) hydrophobic bonds, electrostatic bonds, hydrogen bonds and chelation are the principal attractive forces; (b) electrostatic repulsions and steric hindrance are the principal repulsive forces; (c) a different combination of these forces is established in individual cases by means of the three dimensional geometry of the protein; (d) the amino-acid sequence of the protein plays a key role in establishing this three-dimensional geometry; (e) only a small fraction of the amino-acid residues of the protein are directly involved in this specificity, but some which are distant from the "active site" play a role in establishing the necessary geometry of the essential amino-acids; and (f) the manner in which the individual components combine to determine specificity may include a dynamic interaction in which the small molecule induces a change in the protein conformation as a necessary part of the specificity process. The significance of these features in the interpretation of the specificity patterns of substrates, drugs, haptens and inducers will be discussed.

185 Discussion of the Previous Paper. A. H. THEORELL (Sweden).

186 Structure Activity Relationships among Physiologically Active Peptides. R. SCHWYZER (Switzerland).

Following the break-through achieved in structural elucidation of physiologically active polypeptides by the work of Sanger on insulin, a great number of amino-acid sequences of such products have been elucidated. Amino-acid sequences are obviously determined genetically, being altered according to species and genetic disturbances. The biological activity of polypeptides is mainly determined by amino-acid sequence, the situation being comparable to the transmission of information by the written language. It is of special interest to determine to what extent certain parts of the polypeptide molecule may be subjected to changes without destroying the specific activity, as this may, in the future, give us an idea as to the structure of the certainly chemically related receptor site.

Insight into structure activity relationships has, in the last decade, been obtained by degradational work and, to a larger extent, by synthesis of biologically active polypeptides and analogues containing features different from the natural compounds. The present situation in a few fields, such as ribonuclease, oxytocin and vasopressin, angiotensin, bradykinin, melanocyte stimulating hormones, and corticotropin, will be discussed in detail.

It seems as though certain parts of the molecules are especially important for activity, others less so, and that peptides are as specific in their action as other chemical groups of biologically active substance.

187 Panel Discussion of the Paper of R. Schwyzer: Structure-Activity Relationships among Pharmacologically Active Peptides. R. A. Boissonnas (Switzerland).

A survey of the chemical structures and of the oxytocic, vasopressic and antidiuretic activities of about 50 analogues and homologues of oxytocin and vasopressin, the majority of which were synthesized in the author's laboratory, has shown that some general relations can be found in this class of compounds between the biological specificity and the presence of particular chemical groups.

The relationships between the structures and the CRF activity of several new analogues and homologues of vasopressin are also discussed.

The influence of an N-terminal acetyl group on the specific activity of alpha-MSH and of several related synthetic peptides, prepared in the author's laboratory, is exemplified.

The possibility of structure–activity relationships among several bradykinin analogues and related compounds is debated.

188 Controlled Environment: A Method of Measuring the Drug Response–Behaviour in Humans. J. C. Pollard and L. Uhr (U.S.A.).

Recent advances in techniques have demonstrated the site of action of many psychoactive drugs, while biochemical assay has indicated some of the possible neuro-humoural changes that take place. Animal experimentation with micro-electrodes placed in various selected areas of the brain have demonstrated the locus of drug action and measurement of circulating neuro-humours demonstrate metabolic changes. Behavioural experiments, chiefly using the conditioned response, are also expressly used to define the behavioural manifestations of drug action in animals. While such animal experimentation is appropriate in the initial assay of drugs, the final test of psychoactive drugs must be with human subjects. Situations that are eminently manipulatible with animals cannot readily be used with human subjects, and so many uncontrolled variables make the measurement of drug action difficult.

We have devised a technique for drug evaluation that makes use of the basic "sensory deprivation" procedures to control the environment, both perceptual and socio-emotional, within which behavioural tests of drug effects can be given. We have studied the different effects of several different psychotomimetic drugs (Psilocybin, d LSD 25, and Sernyl) on measures of perception and emotional reactions in subjects tested in this controlled environment. Details of this technique and its initial application are described in the paper.

189 Central Autonomic Reactivity in Humans. (It's alteration by motor activity, adrenaline, and CNS depressing and tranquillizing agents, e.g. amobarbital sodium, Nevental, meprobamate, carisoprodol.) K. Rickels, J. H. Ewing and F. Fochler (U.S.A.).

An inverse relationship between systolic and diastolic blood pressure (BP) rise under cold-pressor (CP) stress and systolic and diastolic BP drop after methacholine injection has been demonstrated recently. The BP response in both tests was felt to depend on central autonomic reactivity. In this study central autonomic reactivity was stimulated by increased proprioceptive input and by injection of adrenaline. CNS reactivity was depressed by 400 mg meprobamate and 700 mg carisoprodol, but not by 200 mg/60 kg amorbital sodium or 100 mg/60 kg Nevental. The theoretical implication of these findings will be discussed in the light of the central action of psychiatric drugs and particularly in the light of Gellhorn's theory of autonomic reactivity.

190 The Action of Miltown on Rats Trained to Concurrent Variable - Ratio Avoidance Schedules. L. Molinengo (Italy).

In the performances of 10 albino rats (5 males and 5 females) on multiple concurrent schedules of avoidance and variable-ratio reinforcements the following parameters were measured: (1) rate of responding on the food re-enforced (right) lever; (2) rate of avoidance responses (left lever); (3) number of avoided electrical shocks; and (4) mean time between the presentation of the warning stimulus and the response (delay).

Differences between subjects and between sexes at different doses of miltown were statistically analysed.

The rates of the food re-enforced and of the avoidance responses were both reduced by miltown. Only the first one, however, showed significant differences between subjects, between sexes and a linear correlation with log doses.

Decrease in the number of avoided shocks was particularly evident with the higher doses; the differences between subjects and between sexes for this last effect were not significant.

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The delay showed an increase particularly with the higher doses of miltown, the differences between sexes and between doses being statistically significant.

These results are tentatively explained in terms of motivational effects.

191 Individual Variations of Pharmacological Central Reactions. G. TONINI and M. BABBINI (Italy).

The variability of pharmacological central reactions, more evident than in other organs, is generally considered a drawback to the elaboration of significant tests. In the higher central functions a number of complex factors play a role, whose summation leads to responses often not accidentally peculiar to the given subject (man or laboratory animal) under study. In the analysis of psychotropic actions the problem of individual variability is by no means to be considered as marginal.

The authors therefore report data obtained through studying general activity and avoidance behaviours in rats. An analysis of motor responses to excitant drugs shows their relation to some parameters of normal activity the type of which may change according to the nature of the drug considered.

The action of caffeine on avoidance behaviours in rats trained with a constant number of tests (reinforcement for every missing conditioned response) was then investigated. The alkaloid administration after suppression of reinforcement results in an increased level of performance. The effect is more remarkable in those animals which had poorer learning results and which showed a prompter tendency to extinction when reinforcements were suppressed.

The authors discuss their data and conclude that the anode of appearance of pharmacological central response variability may represent a useful criterion in characterizing psychotropic drugs.

192 Electroencephalographical Discrimination between Sedato-Hypnotic and Major Tranquillizing Effects in Rats with Non-Extinguishable Conditioned Reflexes. K. KELEMEN, K. MAGYAR and J. KNOLL (Hungary).

Previously it was reported that a non-extinguishable conditioned reflex elaborated in rats with electric shocks of 110 V was highly resistant to even large doses of sedato-hypnotics, but was selectively inhibited by small doses of the major tranquillizers. On the basis of this phenomenon a simple method was proposed for discrimination between the above types of CNS depressants ("jumping test").[1] Recently, it has been shown that in rats with such a non-extinguishable conditioned reflex the conditioned stimulus maintains an extremely long-lasting generalized arousal reaction in the EEG.[2]

In rats conditioned with electric shocks of 110 V and injected with relatively-high doses of sedato-hypnotics (e.g. 40 mg/kg phenobarbital) there occurs only a very limited tendency to the habituation of the generalized arousal reaction evoked by the conditioned stimulus. Low doses of major tranquillizers (e.g. 1·0 mg/kg reserpine) completely inhibit this permanent arousal reaction. The EEG patterns after reserpine treatment do not differ from those of non-conditioned rats and rats with an extinguishable conditioned reflex.

The data confirm the specificity of the "jumping test" in the screening of major tranquillizers and suggest a special state of the brain stem reticular formation in non-extinguishable conditioned reflexes.

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1. KNOLL and KNOLL (1958), *Arzneimittelforschung*, **8**, 330; (1959), *ibid.*, **9**, 663.
2. KELEMEN *et al.*, *EEG Clin. Neurophysiol.* In press.

193 A New Technique for Studying the Inhibitory Effects of Drugs on Discriminatory Avoidance–escape Habits in Rats. C. J. E. J. NIEMEGEERS (Belgium).

A special Warner-type avoidance–escape shuttle box consisting of three identical compartments was used for inducing typical discriminatory avoidance habits in rats, and the inhibitory effects of various drugs on this type of behaviour were studied.

Each experimental session has a constant duration of 5 min and consists of several successive recycling conditions. In order to avoid shock the animal must learn to jump into the green compartment when presented with an auditory warning signal. Selection of the yellow compartment or failure to make an avoidance response within 10 sec is punished with shocks from the grid. The animals must also learn not to leave the safe green compartment unless presented with the next warning signal.

After about 50 daily training sessions about a third of the rats are adequately trained, i.e. succeed in avoiding punishment consistently by continuously making the appropriate discriminatory avoidance response.

It was found that a series of neuroleptic agents chemically related to chlorpromazine or to haloperidol are selective inhibitors of the avoidance-habits of these adequately trained rats at dose levels not producing overt signs of neurological impairment. Failure to discriminate is observed only at significantly higher dose levels.

194 The Action of Some CNS Depressants and Stimulants on the "Carried" Extinction in Rats. E. BECCARI and L. MOLINENGO (Italy).

After rats have been conditioned in a double lever Skinner box to obtain reinforcements at a fixed ratio schedule from one lever only, reinforcements are disconnected from the lever the rats have

been trained to operate on and connected to the other one.

The development of a positive drive for the last lever "carries" on the drive extinction for the former, so that complete reconditioning occurs sooner than in ordinary extinction. Furthermore, the adjustment to the new conditions may be repeated in successive trials, in which case some degree on facilitation occurs and a change in shape of the extinction curves has been observed.

Attempts to fit these curves by an unitary analytical formula failed to yield satisfactory results, because of several factors involved.

Owing to the lever location in the cage, changes from right to left or from left to right are not equivalent, the steady state in the extinction curves being reached more easily when the reinforced lever is nearer to the food tray. Differences were also observed between males and females, the former responding with a higher rate on both levers.

The action displayed by some CNS depressants (Phenobarbital, Chlorpromazine, Miltown) and stimulants (Amphetamine) has been investigated at three dose levels for each drug, using a total of 8 animals of both sexes. The rate of change in the responding activity may be accounted for by the psychopharmacological properties of the drugs tested.

195 Assessment of Pharmacological Actions upon the Brain, Based upon Experimental Studies of Habituation. E. M. Glaser (United Kingdom).

Habituation is the gradual diminution of responses to repeated stimuli. In man it can be inhibited by chlorpromazine hydrochloride,[1, 2, 3] and there may be some similarity between the actions of this drug upon disorders of the mind and upon habituation. This can be studied further in rats by a method which involves first the training of conscious rats to the experimental situations (including immersion of their tails in water at 31 °C), and then repeated immersions of the tails to a constant level in water at 4 °C. Automatic counts of the rats' electrocardiographic impulses are made before and during immersions of the tails at 4 °C. During cooling of the tails the heart rate rises about 10–20 per cent above its level at 31 °C, but if the tails are immersed several times daily at 4 °C for about 10 days, then this increase of the heart rate becomes gradually less. That such a lessening of the rise of heart rate is brought about by the brain was shown experimentally by the fact that rats with bilateral superficial lesions of the frontal areas of the brain showed a constant increase of the heart rate during repeated immersions of the tail at 4 °C over 10 days, although rats with lesions elsewhere on the brain surface behaved very similarly to unoperated controls.[4] This method thus allows quantitative assessment of a reproducible response to a precisely defined stimulus, and it can be used to study drug actions both upon responses in unhabituated animals and upon habituation. Moreover, because of the relationship of habituation with specific areas of the brain, this technique can sometimes show how certain drugs act upon the brain.

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196 Studies on the Functional State of Brain in the General Action of Procaine. G. I. Zobcallo and T. M. Kuchierenko (U.S.S.R.).

Experiments were carried out on dogs and rabbits. Procaine was administered subcutaneously to ensure a slow entry into the bloodstream and gradual development of the general action. The results were as follows:

(1) The general clonic and tonic convulsions in rabbits evoked by large doses of procaine (150–200 mg/kg) depend on the effect of this drug on diencephalon.

(2) Investigation of the conditioned reflex activity in dogs during the general action of procaine revealed that the top centre of the brain were the most susceptible part of the central nervous system to this drug. Small doses of procaine (1–2 mg/kg) elicited a stimulating effect with an increase of the positive conditioned reflexes; large doses of procaine (10–20 mg/kg) produced a depressant effect.

(3) Studies of the effect of constituents of procaine on the conditioned reflex activity revealed a stimulating effect from *p*-aminobenzoic acid and a depressant effect from diethylaminoethanol. Both substances proved to be much less active than procaine. These experiments showed that the effect of procaine on conditioned reflex activity was due to its unsplit molecule.

(4) In experiments on dogs treated with sulphanilamide, it was found that both *p*-aminobenzoic acid and procaine failed to stimulate conditioned reflex activity. Thus with regards to the functions of highly developed cerebral nerve cells competitive antagonistic relations were established between sulphanilamide and *p*-aminobenzoic acid or procaine similar to those previously demonstrated with these drugs in their action on certain bacteria.

197 The Effects of Vitamin B_{12}, B_6 and Procaine on the Conditioned Escape Response. R. Kiesewetter, R. Itterheim and J. Gebhard (Germany).

Rats from the Wistar-strain were trained to learn a conditioned response with a buzzer as conditioned stimulus and an electric shock as the unconditioned

stimulus. The stimulus-schedule was that of Knoll and Knoll. In the first 10 days (first period) the conditioned stimulus was reinforced with the electric shock. In the following period (second period) the conditioned stimulus was given alone. In one group, each animal was given 100 μg/kg Vitamin B_{12} i.p., in a second 50 mg/kg Vitamin B_6 i.p., in a third 25 mg/kg procaine subcutaneously and in a fourth 0·5 ml NaCl-solution. The results were statistically analysed by the Kruskal–Wallis test. The latent period of Vitamin B_{12}-treated animals was not any different from that of animals treated with the other substances or the controls in the first training period. The number of negative responses however is smaller after Vitamin B_{12}. In the second traing period there was a significant increase in the latent period and the number of negative responses. Vitamin B_6 and procaine have no effect in comparison with NaCl-treated controls. All the Vitamin- and procaine-treated animals showed a great increase in body wt. (nutrition-effect). The effect of Vitamin B_{12} is, as in our earlier investigations on the suppression of strychnine-induced convulsions and the isolation-induced aggression-behaviour, like that of a weak tranquilizing agent.

198 Pharmacological Observations on the Responses to Afferent Stimulation of the Vagus Nerve. J. A. Izquierdo, A. J. Kaumann, J. D. Coussio and I. Izquierdo (Argentine).

We studied the responses to afferent stimulation of the vagus nerve (A.S.V.) in 135 dogs. The following effects were recorded: (1) vascular effects (pressor, depressor, biphasic or no responses); (2) ECG effects in 28 dogs: bradycardia (46 per cent), tachycardia (14 per cent), modifications of (70 per cent); (3) inspiratory apnoea; (4) increased anaesthesia; and (5) changes in duodenal motility (15 dogs).

T-wave Pressor responses were enhanced by cocaine, diphenhydramine, serpentine, imipramine and pirogalol; attentuated by reserpine (inversion), adrenergic and ganglionic blocking agents and adrenalectomy.

Depressor responses were enhanced by reserpine and attenuated by imipramine (in acutely reserpinized dogs) and diphenhydramine.

Tachycardia and changes in P-Q, S-T and T-wave similar to those produced by norepinephrine were more apparent after imipramine.

Inspiratory apnoea was apparently unmodified by the afore-mentioned drugs.

According to our results, A.S.V. effects may be due to activation of central regulatory areas of the sympathetic tonus, excluding the area postrema (whose destruction did not alter A.S.V. responses) and those areas involved in the carotid occlusion reflex.

This central activation would provoke peripheral and adrenal liberation of norepinephrine (as evidenced by ECG and imipramine) and epinephrine (as evidenced by pirogalol and eventually by ECG).

In our experience, angiotensin, 5-HT and vasopressin are not responsible for A.S.V. responses (ECG and blood pressure studies).

Variations in our results may be ascribed to previous differences in the central regulation of the sympathetic tonus of dogs, which may be influenced by a number of factors: anaesthesia, age, sex, etc.

199 Effects of Drugs on Timing Behaviour. G. Maffii and D. Costantini (Italy).

The effects of amphetamine, caffeine, atropine, pentamethylenetetrazole, diethyloxazinedione, β-phenylisopropylhydrazine, iproniazid, LSD_{50}, 5-phenyl-2-imino-4-ketooxazolidine and phenylcyclopropylamine, have been studied on timing behaviour of conditioned rats. The experimental situation was that described by Sidman and termed "differential reinforcement of low rates". In brief: the animal must space his response in time if he is to secure the reward. In our condition a lever-pressing response produced the reinforcement only if it followed the preceding lever-pressing after an interval of at least 20 sec.

The data have been analysed by plotting the probit of per cent cumulative responses "emitted" in every 2 sec interval after each reinforcement, against the log of the time between two reinforcements and then calculating the time corresponding to the maximal response rate (and its confidence limits). The changes in patterns of behaviour produced by the mentioned agents may be summarized as follows:

Amphetamine—1–2 mg/kg orally produces a slight anticipation of the maximal frequency of responses; this effect is more marked after 2·5 mg/kg. The distribution however appears regular and the number of responses, for a 2 hr interval, is increased.

Phenylcyclopropylamine—at 10 mg/kg orally produces similar changes, and tends to shorten the inter-response times. However the maximum effect is reached 15 hr after administration. No effect was detected with a dose of 2·5 mg/kg.

β-Phenyl-isopropylhydrazine—at 5 mg/kg orally does not significantly affect the timing behaviour, while at 20 mg/kg depressed the total output and almost completely does away with all evidence of timing behaviour.

Caffeine—in oral doses from 1·25–10 mg/kg does not produce any apparent changes.

Pentamethylenetetrazole and dioxone—are similarly ineffective and only in doses of 20 and 5 mg/kg (respectively) produce a decrease of responses.

Atropine—0·2 mg/kg (rally) does not produce any apparent effect and at 1 mg/kg slightly flattens the response distribution.

Iproniazid—at 20 mg/kg/day i.p. for three days decreases the total output and makes the distribu-

tion irregular; a single 50 mg/kg i.p. dose does not apparently affect the timing behaviour in the 18 hr following administration.

LSD_{50}—is ineffective at 0·1 mg/kg orally; at 0·5 mg/kg it completely does away with all evidence of timing behaviour.

The preliminary data here presented, indicate that only some drugs strictly related by their chemical structure are able to produce typical changes of the "spaced responding" without either depression of the total output or abolition of timing behaviour.

200 Enumeration of Catecholamine—containing Heavy Granules of Adrenal Gland. W. R. Burack, P. R. Draskoczy and N. Weiner (U.S.A.).

A method has been developed for counting catecholamine-containing heavy granules isolated by gradient density centrifugation[1] of the large granule fraction prepared from a homogenate of fowl adrenal gland. The heavy granule pad is gently rehomogenized in a solution of 1·8 M sucrose containing 0·01 M EDTA and an aliquot is mixed with a measured volume of counted, washed red blood cells which have been similarly suspended. A drop of the mixture is placed on a glass slide and observed through a glass coverslip under oil-immersion with a phase-contrast microscope (2500 × magnification). By simultaneously counting the red cell and heavy granule elements, an estimate of the number of the latter may be obtained. The total number of heavy granules recovered in pads from 6 normal pairs (mean wt. 200 mg) averaged $1{\cdot}1 \times 10^{10}$ (range $0{\cdot}49$–$1{\cdot}76 \times 10^{10}$). Correlation of counts with biochemical data and with electron micrographic determinations of heavy granule dimensions reveals in each case an intragranular concentration of catecholamines well in excess of one millimole per millilitre of granule. Unless one assumes an unusually high resistance to osmotic forces on the part of the heavy granule, these findings reinforce the view that catecholamines are largely in the bound form within the granule. This method of counting has also been applied to adrenal glands of reserpine-pretreated birds.

(Supported in part by U.S. Public Health Service Grants H-2203 and H-3674; American Heart Association Advanced Fellowship 59 AF 91; and U.S. Air Force SAM Contract AF 41 (657)-197.)

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201 Effect of Some Drugs on Noradrenaline Release from Nerve Granules. U. S. von Euler and F. Lishajko (Sweden).

Transmitter granules containing noradrenaline can be isolated from homogenates or press juice of adrenergic nerves.[1, 2] Of the total activity in bovine splenic nerves, on an average, 28 per cent was found in the granules. The granules show no loss of noradrenaline over many hours in isotonic neutral solutions of K-phosphate at 0–4°C, but rapidly lose their contents on addition of acids, detergents and certain drugs, or when exposed to 37°C or higher temperature.

Reserpine releases noradrenaline when added in concentrations of 0·1 mg/ml or more to resuspended granules. However, during incubation at 20°C the spontaneous release of noradrenaline is almost completely blocked by reserpine 1–10 μg/ml, added directly to the granule suspension. Partial block is noted even with 0·01 μg/ml,[3] if the granule suspension is pretreated with reserpine for 30 min at + 4°C. The releasing effect of tyramine[4] is prevented by reserpine 1–10 μg/ml, but not by 0·01–0·1 μg/ml. The blocking of NA release in oligodynamic doses is believed to be the underlying factor for the NA depletion of organs, assuming that resynthesis and/or uptake is similarly blocked.

Blocking, though less complete, has also been observed for cocaine, xylocaine, TM-10 and phenoxibenzamine, but not for guanethidine. In higher concentrations cocaine, xylocaine and TM-6 increase the rate of release of noradrenaline from nerve granules, like promethazine and catechol, while acetylcholine, 5-HT and nicotine appear to be without action.

Storage granules from the adrenal medulla of the rabbit show a slower rate of spontaneous release than nerve granules. The rate of release can be greatly enhanced by administering dopa to the animal.

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202 The Local Release of an Adrenaline-like Substance during Inflammation. D. A. Willoughby and W. G. Spector (United Kingdom).

We have previously shown that the administration of mono-amine-oxidase (M.A.O.) inhibitors greatly lessens the increased capillary permeability that occurs in rat skin after thermal injury.

We have now extended our observations to chemical and radiation injury. Pretreatment of rats with M.A.O. inhibitors leads to a marked diminution of increased capillary permeability after all these forms of injurious stimuli. The mode of action of the M.A.O. inhibitors is thought to be the preservation of a substance closely resembling adrenaline released locally during injury. Thus the inflammatory action of these compounds can be imitated by systemically administered adrenaline, but not by 5-hydroxytryptamine, or noradrenaline.

In addition, the effect of the M.A.O. inhibitors may be reversed by means of a systemically-administered adrenolytic (dibenamine) but not by low doses of lysergic acid and its derivatives antagonistics to 5-hydroxytryptamine.

No inhibition of increased capillary permeability was observed after pretreating animals with pyrogallol, the inhibitor of catechol-*o*-methyl transferase, or after treatment with adrenochrome, normetanephrine, or 3-methoxy-4-hydroxy-mandelic acid (V.M.A.).

The inactivation of this vasoconstrictor amine apparently proceeds throughout the period of increased capillary permeability.

Immediate local application of M.A.O. inhibitors after thermal injury is followed by a marked suppression of inflammatory oedema.

203 The Mechanism of Catecholamine Release by Tyramine. H. J. Schümann and A. Philippu (Germany).

The indirect sympathomimetic action of tyramine is assumed to be mediated by a liberation of noradrenaline and adrenaline. In the suprarenal medulla as well as in sympathetic (splenic) nerves these catecholamines are mainly stored together with ATP in specific elements of the cell, the chromaffin granules. When isolated chromaffin granules from suprarenal medulla of cattle are incubated in isotonic sucrose-phosphate buffer of pH 6·8 at 37°C in air, a spontaneous release of the stored catecholamines along with ATP takes place. As the percentage decrease of both catecholamines and ATP is nearly equal, the molar ratio amine/ATP remains unchanged. By addition of tyramine, the spontaneous release of catecholamines is enhanced whereas the ATP release is not. Therefore, the molar ratio amine/ATP is changed to lower values. The amount of the released catecholamines under the influence of tyramine is replaced stoichiometrically by an uptake of tyramine. When tyramine causes no liberation of catecholamines—incubatio at 0°C—no uptake of tyramine by the granules can be observed.

Experiments with isolated perfused suprarenals have shown that tyramine is able to release catecholamines also from intact cells.

The results are discussed with respect to the mechanism of the action of tyramine as well as of other "indirectly" acting sympathomimetic amines.

Growth and differentiation of the sympathetic system have been shown to be under control of a specific protein (N.G.F.). Injection of an antiserum to the N.G.F. in newborn mammals nearly totally destroys the sympathetic ganglia.[1] As a result of this treatment one can obtain large colonies of adult animals without sympathetic systems. The purpose of this investigation was to compare the content of sympathetic transmitters and of monoamine-oxidase (MAO) activity at the peripheral level in control and immunosympathectomized animals.

204 Effect of Tyramine and Meta-Tyramine on Perfused Ears from Reserpinized Rabbits. F. P. Ludueña (U.S.A.).

Excised ears were perfused with Krebs–Henseleit solution at 34°–35°C. In preparations from unmedicated rabbits, *m*-tyramine (3-OH-phenylethylamine, *m*-T) and L-norepinephrine (NE) were approximately 5–10 and 250–500 times more vasoconstrictive than tyramine (T), respectively. In preparations from reserpinized rabbits (3 mg/kg, subcutaneously daily for 1 to 5 days) the *m*-T/T and NE/T activity ratios were larger, mainly because of increased sensitivity to *m*-T and NE. In unmedicated and reserpinized rabbit ears, perfused for 2 or 3 consecutive days, but kept overnight at 4°C without perfusion, repeated injections (30–38) of T continued to produce undiminished intense vasoconstriction.

Experimental results reported from various laboratories tend to support Burn and Rand's hypothesis, that tyramine acts indirectly, by the local release of stored NE. According to these investigators, the mean NE content of the reserpinized rabbit ear is approximately 0·03 μg/g (0·6–0·7 per ear). It would be difficult to explain the results reported above by assuming that the effect of 38 injections of T made over a period of 3 days was dependent on such amount of stored NE. In addition, tachyphylaxis did not occur on repeated injection, which would have been expected if the effect of T depended on a constantly decreasing amount of stored NE. It is concluded that an alternative mechanism is required to explain the action of tyramine on the rabbit ear.

205 Quantitative Aspects of the Restoration of Vasoconstrictor Effects in Reserpinized Cats. S. Rosell and G. Sedvall (Sweden).

Reserpine 5(mg/kg) causes a complete inhibition of the effects of vasoconstrictor nerve activity. The vasoconstrictor effects can be restored by infusion of doa, dopamine, nor-adrenaline and adrenaline.[1, 2] Important quantitative differences in the vasoconstrictor responses exist however.

Vasoconstrictor effects in skeletal muscle vessels could be evoked by sympathetic chain stimulation at least one hour after the intra-arterial infusion of nor-adrenaline had been finished. The same was true after dopa and dopamine infusion but not after administration of adrenaline. The threshold doses for dopamine and nor-adrenaline were 25 μg, for dopa 2 mg and for adrenaline 100 μg. The frequency–response curves obtained on stimulating the sympathetic chain and measuring blood flow in a muscle region after intra-arterial infusion of dopa, dopamine, nor-adrenaline and adrenaline differed significantly from the frequency–response curve obtained from control animals, i.e. animals which had not been treated with reserpine and in which no

infusion of the mentioned substances had been performed.

1. Burn, J. H. and Rand, M. J. (1958), *J. Physiol.*, **144,** 314.
2. Rosell, S. and Rosén, A. (1961), *Acta Physiol. scand.*, **52,** 53.

206 The Effect of Drugs on the Uptake of Isotopic Norepinephrine in Various Tissues. H. J. Dengler and E. O. Titus (Germany).

Isotopically labelled catecholamines enter slices of cat brain, heart or spleen both by simple diffusion and by a transport mechanism. The latter, which accounts for most of the inward flux in media containing 5–25 mμg/ml of norepinephrine, is inhibited almost completely by 10^{-6} M reserpine, cocaine, chlorpromazine, guanethidine and tofranil and by 3×10^{-5} M bretylium. Recanescine and rescinnamine are approximately as inhibitory as reserpine, but the physiologically inactive *iso*reserpine is without effect. Ergotamine, regitine, dichloro*iso*proterenol, tyramine, ephedrine, amphetamine and, mescaline are partially inhibitory. Dibenamine; acetyl choline, lysergic acid and serotonin are inactive. Many of these inhibitory compounds enhance pharmacological responses to norepinephrine. It is possible that the concentrating mechanism is a means for terminating physiological activity. Heart slices from rats pretreated with thyroxine (also a sensitizer to norepinephrine) took up 70 per cent as much isotope as controls incubated with norepinephrine-^{3}H. Ten minutes after injection of 6·6 μg/kg of this amine, radioactivity in the hearts of thyroxine-treated rats was 37·5 per cent of controls. In normal hearts the following compounds accounted for these per cents of total isotope: norepinephrine, 37·3; epinephrine, 19·6; normetanephrine, 17·6; acidic products of amine oxidase, 6·1. Corresponding percentages after thyroxine were: 92·1, 0, 4·1 and 2·1.

207 Modification of Noradrenaline Uptake by Dibenzyline. J. A. Harvey and J. N. Pennefather (Australia).

Recent work has led to suggestions that uptake of noradrenaline by tissues represents one step in the physiological disposition of this amine.[1, 2] We have examined the effects of single doses of the adrenergic blocking drug, dibenzyline, on this uptake of noradrenaline in the cat. The results obtained point to a selective modification of noradrenaline uptake by dibenzyline. In the kidney, where the action of noradrenaline is excitor, uptake was completely prevented, but in the uterus, where the action of noradrenaline is inhibitory, uptake remained unaffected.

Since prolonged treatment with dibenzyline has been shown to cause catecholamine depletion,[3] some pharmacological consequences of this procedure have also been investigated. Pressor responses to injected adrenaline, noradrenaline and tyramine were found to be modified by this treatment. Differences in the actions of these amines revealed by acute dibenzyline treatment, chronic dibenzyline treatment and treatment with reserpine, will be discussed.

1. Axelrod, T., Whitby, L. G. and Hertting, G. (1960), *Nature* (*Lond.*), **187,** 604.
2. Pennefather, J. N. and Rand, M. J. (1960), *J. Physiol*, **154,** 277.
3. Shapiro, S. (1958), *Acta Physiol. Scand.*, **42,** 371.

208 Adrenaline-Noradrenaline Uptake in Rat Organs. B. C. R. Strömblad (Canada).

It is well-known that following sympathetic denervation, the catecholamine content of an organ is greatly reduced, noradrenaline more so than adrenaline.

In the present experiments no noradrenaline could be detected in the submaxillary and parotid glands of the rat one week after excision of the superior cervical ganglion while some adrenaline was still present. When the suprarenal medullae were removed in addition to denervation of the salivary glands no catecholamines could be detected. Demedullation alone did not change the catecholamine content of the salivary glands or the heart.

From these experiments it was concluded that the adrenaline stored in cells other than the sympathetic nerves could be derived either from material synthesized in the sympathetic nerves or in the suprarenals.

The ability of rat organs to accumulate catecholamines from the blood was therefore studied further.

One hour after the injection of 1 mg noradrenaline into previously demedullated rats the noradrenaline content of the heart and of the submaxillary and parotid glands was greatly increased (2–3-fold). If the superior cervical ganglion had been excised one week previously, only small amounts of noradrenaline were found in the salivary glands.

One hour after injection of 1 mg adrenaline the adrenaline content of the heart and salivary glands was high (up to 10 times normal) and the noradrenaline content was decreased in the heart and submaxillary gland (1/3–2/3 normal). If the superior cervical ganglion had been removed one week previously, the adrenaline content after injections was low, of the same magnitude as those for noradrenaline in the similar experiments. Furthermore, the adrenaline or noradrenaline contents of denervated glands after catecholamine injections were similar to the adrenaline contents of denervated glands of animals with intact suprarenals.

209 The Effects of Drugs on the Noradrenaline Uptake into the Heart. E. Muscholl (Germany).

The effects of drugs, known to modify the action of sympathomimetic amines, on the noradrenaline uptake into the heart were studied in the pithed rat. Noradrenaline (5–20 μg per rat in 20 min) was infused intravenously and the heart excised 5 min later. Infusion of 5 and 20 μg of noradrenaline caused an increase in heart noradrenaline concentration by 39 and 69 per cent, respectively.

Reserpine[1] and cocaine[2, 3] are known to block the noradrenaline uptake into the heart. The specificity of the action of cocaine (0·15–20 mg/kg) has been investigated. With infusions of 5 and 20 μg of noradrenaline, the doses of cocaine needed for 50 per cent inhibition of net uptake of noradrenaline were 0·7 and 10 mg/kg, respectively.

For a given dose of cocaine, there was a significant correlation between increase of the pressor response to a test dose of noradrenaline and inhibition of the noradrenaline uptake into the heart.

Drugs chemically related to cocaine such as alpha-cocaine, tetracaine and atropine (10–20 mg/kg), did not alter the noradrenaline uptake or cause supersensitivity towards noradrenaline. Noradrenaline uptake was unchanged after dibenamine (50 mg/kg), but completely blocked by dichloroisoprenaline (DCI, 30 mg/kg).

It is concluded that injected noradrenaline not only combines with adrenergic receptors but is stored at other sites as well. Cocaine prevents uptake into these sites, thereby increasing the amount of noradrenaline available for combination with adrenergic receptors. DCI blocks both the uptake into stores and the combination with receptors.

1. Muscholl, E. (1960), *Arch. Exp. Path. Pharmak.*, **240,** 234.
2. Muscholl, E. (1960), *Ibid.*, **240,** 8.
3. Axelrod, J., Whitby, L. G. and Hertting, G. (1960), *Nature* (*Lond.*), **187,** 604.

210 A Species Difference in Metabolism of Myocardial Catecholamines. F. E. Shideman and N. D. Goldberg (U.S.A.).

We previously demonstrated a depletion of myocardial catecholamines (MCA) in one species (cat) and an increase in concentration of MCA in another species (rat) *in vivo* by the monoamine oxidase inhibitor (MAOI), 2-*trans*-phenylcyclopropylamine (SKF 385). A positive inotropic effect is produced by SKF 385 on isolated atria of both species. This action is blocked by pretreatment with dichloroisoproterenol or trimethyl (2-(2:6-xylyloxy)-propyl)-ammonium chloride and abolished or greatly reduced in preparations from reserpine-treated animals. This evidence suggests the inotropic response is effected by a release of catecholamines (CA) in both species. It is conceivable that the comparable physiological effects of SKF 385 on heart muscle from the cat and rat and the observed difference in response of MCA to administration of this MAOI might be explained by a difference in the metabolic mechanisms available for its biotransformation in the two species. A study of the *in vitro* metabolism of norepinephrine (NE) by heart homogenates disclosed a rapid rate of metabolism by rat heart but little or no metabolism by cat heart unless ATP and L-methionine were added. These additions did not enhance metabolism of NE by rat heart. Disappearance of NE in rat heart homogenates containing added ATP and L-methionine was inhibited by SKF 385 but not affected significantly by catechol. SKF 385 did not inhibit metabolism of NE by cat heart homogenates supplemented with ATP and L-methionine but catechol did. These results indicate that the predominant CA inactivating enzyme in the myocardium of the rat is MAO and in the cat is catechol-O-methyl transferase.

211 The Potentiation of Adrenaline and Noradrenaline by Cocaine on the Innervated and the Denervated Nictitating Membrane of the Cat. W. R. Kukovetz and F. Lembeck (Austria).

Consecutive doses of 0·1–10·0 ug/kg adrenaline and noradrenaline were injected intravenously to spinal cats. Contractions of both nictitating membranes one of which had been denervated one week prior to the experiment were recorded. Subsequently, 3 mg/kg of cocaine were given and the same doses of catecholamines were injected again. On the innervated membrane the well-known potentiation of the catechol effect by cocaine occurred. On the denervated nictitating membrane, however, cocaine completely failed to potentiate the effect of adrenaline or noradrenaline.

These results are in agreement with the observation of Hertting *et al.* (1961) who found that denervated tissue did not take up ^{3}H-noradrenaline. Cocaine, which presumably exerts its potentiating effect on the action of catecholamines by blocking their unspecific receptor sites around the nerve endings and hereby making available more catecholamines to the specific receptors, would as a result of the denervation be deprived of its substrate and, therefore, not be able to produce potentiation.

212 The Influence of Reserpine on the Reactivity of Isolated Rabbit Aorta and Carotid Artery. C. N. Gillis and C. M. Yates (Canada).

Pretreatment of rabbits on each of 2 days before use with reserpine (2 mg/kg i.p. first day and 2 mg/kg i.v. second day), completely abolished the secondary contraction of rabbit aorta and carotid artery which normally follows the cessation of an

alternating current stimulus (10 or 15 V. a.c. for 10 sec). The adrenergic blocking agent Piperoxan (0·5 μg/ml) was found to have a similar, but reversible blocking effect on the secondary contraction, thus confirming the observations of Furchgott.[1] Estimation of the catecholamine content of aorta from reserpine-pretreated rabbits revealed significantly lower values than those of aorta from untreated animals. This depletion of catecholamine, previously reported by Burn,[2] appears to be produced rapidly, since we have found that it can be observed even 2 hr after the administration of 2 mg/kg reserpine i.v. At this time, the secondary response to electrical stimulation was also completely abolished.

Tolazoline hydrochloride, in doses smaller than those causing adrenergic blockade, produced prolonged contractions of aorta. The sensitivity of rabbit aorta to tolazoline could be increased 10–20 times by reserpine pretreatment. There was, in fact, a parallel increase in the sensitivity of aorta to norepinephrine and tolazoline, at a time when no secondary contraction, following electrical stimulation, could be elicited. Therefore, the presence or absence of this response to a.c. stimulation may form a sensitive indication of the state of the stores of catecholamine in the tissue.

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1. Furchgott (1952), *Fed. Proc.*, **11**, 217.
2. Burn and Rand (1958), *Brit. med. J.*, **1**, 903.

213a Variations dans la Teneur en Adrénaline du Liquide Ayant Perfusé l'Oreille Isolée de Lapin I-Etude de l'Action des Adrénalino-Sensibilisateurs. R. Hazard, M. Beauvallet, E. C. Savini et S. Larno (France).

Des recherches récentes ont démontré que l'adrénaline administrée sous forme d'infusions intraveineuses continues disparait très rapidement du plasma. Afin de suivre la destinée de l'adrénaline dans les tissus on expérimente sur l'oreille isolée de lapin, ce qui limite les facteurs intervenant chez l'animal entier.

L'oreille isolée de lapin est perfusée à l'aide d'un soluté physiologique salé contenant de l'adrénaline ou bien un mélange d'adrénaline avec éphédrine, cocaïne ou iproniazid.

L'effet vasoconstricteur est mesuré en gouttes par minute et la quantité d'adrénaline dans le perfusat est dosée par fluorométrie; trois mesure sont faites: (1) au début; (2) lors du maximum; et (3) pendant la diminution de l'action.

Les perfusions à adrénaline provoquent une vasoconstriction peu durable avec utilisation (destruction ou fixation), dès le début de la moitié de la quantité de neurohormone perfusée. Au moment de l'action maximale la quantité d'adrénaline utilisée est plus réduite car il y a saturation des récepteurs. Pendant la diminution de la vasoconstriction cette quantité n'est que de 24 pour cent seulement par rapport à la concentration dans le liquide de perfusion.

L'éphédrine et la cocaïne renforcent la vasoconstriction plus qu'elles ne la prolongent, tandis que l'iproniazid la prolonge très nettement.

Le rapport entre la quantité d'adrénaline tissulaire fixée (éventuellement libérée par la tyramine) et celle détruite nous renseignera-t-elle sur l'influence des différents facteurs biochimiques et pharmacologiques dans la vasoconstriction des vaisseaux? La discussion porte sur le rôle de ces facteurs (adrénalino-sensibilisateurs, aminoxydase et son inhibiteur l'iproniazid, et 3-0-méthyltransférase et son inhibiteur le pyrogallol).

213b Variations in the Amount of Adrenaline found in the Outflow Fluid of the Isolated Rabbit Ear. I. The effects of adrenaline-sensitizing substances. R. Hazard, M. Beauvallet, E. C. Savini and S. Larno (France).

Recent work has shown that the continuous intravenous infusion of adrenaline disappears quickly from the plasma. In order to follow the fate of adrenaline in a less complicated system than the whole animal, assays were made on the isolated rabbit ear preparation.

The ear was perfused with a physiological saline solution containing adrenaline or a mixture of adrenaline with ephedrine, cocaine or iproniazid. The outflow during the vasoconstrictor effect was measured in drops per minute and the concentration of adrenaline in the outflow fluid was estimated by fluorimetry. Three determinations were made, the first at the beginning, the second at the time of maximum vascular response and the third during the decrease of the action.

At the start of the perfusion, there is a transitory vasoconstriction: only about half of the adrenaline comes out in the perfusate. At the time of maximum vasoconstriction, less adrenaline is taken up or destroyed by the tissues, presumably because of receptor saturation. During the decrease of vasoconstriction only about 25 per cent of the adrenaline is removed by the tissues.

Ephedrine and cocaine potentiate the vasoconstriction, whereas iproniazid obviously prolongs it.

It is possible that the ratio between the amount of adrenaline retained by the tissues (eventually liberated by tyramine) and that destroyed may throw light on the role in vasoconstriction of the different biochemical and pharmacological factors. The actions of adrenaline-sensitizing drugs, amine oxidase and its inhibitor iproniazid, catechol-O-methyltransferase and its inhibitor pyrogallol will be discussed.

214 Effects of Cocaine on the Central Nervous System. E. Eidelberg, H. Lesse and F. P. Gault (U.S.A.).

It has been found that cocaine, at a dose range of 10–20 mg/kg in cats, induces marked changes in the electrical activity recorded from the amygdaloid complex. Depending on the dosage these changes range from increase in the high voltage, 40 c/s, rhythmic pattern characteristic of this region, to convulsive discharges. These may be followed by generalized seizures. Equivalent effective dosages in rats are larger by a factor of 3 to 4. In both species the concomitant behavioural changes strongly resemble those of "psychomotor epilepsy".

Studies on rats have shown that previous bilateral amygdaloid destruction prevents the appearance of seizures after a convulsant dose of cocaine. Rats are significantly protected against cocaine-induced convulsions by chlorpromazine, reserpine, dibenamine and pyridoxine. Pretreatment with monoamino-oxidase inhibitors potentiates the convulsant actions of cocaine, while inhibition of catechol-0-methyltransferase does not.

It is proposed that cocaine acts selectively on brain structures employing an "adrenergic" type of transmission. On the basis of these data it is also proposed that serotonin may be an important factor in transmission in the amygdaloid complex.

215 Relationship between Anti-Arecolin Activity and Potentiation of the Pseudo-narcotic Action of Morphine by Atropine Substitutes. A. Herz (Germany).

Arecoline reduces the reaction to nociceptive stimulation in animals; this effect can be abolished by atropine-like substances possessing central activity. Quantitative proof of the anti-arecoline potency of atropine substitutes is obtained by employing Haffner's method of testing anaelgesia in mice.(1) Apart from this, centrally acting atropine substitutes potentiate the morphine-induced pseudo-narcosis in the rat. A method has been developed to establish this potency quantitatively.(1)

The activity of about 20 substances possessing more or less marked atropine properties has been investigated in both tests; it was found that there exists a distinct correlation between the anti-arecoline activity and the morphine-pseudo-narcosis potentiating activity of the substances. Most active is scopolamine; it is followed in some distance by hyoscyamine and antiparkinsonian compounds, i.e. benactyzine, trihexyphenidyl, akineton, procyclidine, caramiphen and others; low activity or none at all was observed, for example, in substances like diphenhydramine and orphenadrine and also in the dextro-rotary isomers of optidally-active atropine substitutes. The effective doses of the investigated substances differ by nearly 10^2. From these results it is suggested, that the potentiation of the narcotic equivalents of morphine activity by atropine substitutes originates in the inhibition of a central cholinoceptive system.

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1. Herz, A. (1961), *Arch. Exp. Path. Pharmak.* In press.

216 The Combined Action of Morphine and Atropine. R. Eerola (Finland).

There are greatly differing opinions in the literature concerning the combined action of morphine and atropine given simultaneously. The earlier workers considered the joint action of these drugs antagonistic, later investigators reported that atropine enhanced the action of several central depressant drugs. The author's own experiments in white mice indicate that the combined action of morphine and atropine is synergistic, the type of synergism being additive. The criterion was lethal toxicity. The results will be presented mathematically.

217a Der Einfluß von Morphin und Laevallorphan auf Motilität, Sauerstoffverbrauch und Rektaltemperatur sowie den Gehalt des Gehirns der weißen Maus an Kreatinphosphat, ATP, ADP, Milchsäure, Glykogen und Coenzym A. C. J. Estler (Germany).

An weißen Mäusen wurde eine Stunde nach Verabreichung von Morphin und Laevallorphan (3-Hydroxy-N-Allylmorphinan) einzeln und in Kombination der Einfluß auf die Spontanmotilität, den Sauerstoffverbrauch, die Rektaltemperatur und den Gehalt des Gehirns an Kreatinphosphat, ATP, ADP, Glykogen, Milchsäure und Coenzym A untersucht. Laevallorphan in einer Dosierung von 0·5, 1·0 und 5·0 mg/kg s.c. führte zu keiner signifikanten Änderung der untersuchten Stoffwechselgrößen und der Motilität. Fünfzehn mg/kg Morphin s.c. riefen eine Steigerung der Spontanmotilität auf das 18-fache der Norm, sowie eine Senkung des Sauerstoffverbrauchs um 12 Prozent und eine Herabsetzung der Rektaltemperatur um 2·2°C hervor. Im Gehirn stieg unter Morphin der ATP-Gehalt von 1·94 auf 2·17 µM/g, der Gehalt an ADP sank um annähernd den gleichen Betrag ab, der Kreatinphosphat-Gehalt fiel von 3·24 auf 2·67 µM/g, die Milchsäure von 1·49 auf 1·19 µM/g. Der Glykogen- und Coenzym-A-Gehalt blieben unverändert. 1·0 und in schwächerem Maße 0·5 mg/kg Laevallorphan wirkten gegenüber Morphin antagonistisch. So zeigten 1 Stunde nach gleichzeitiger Verabreichung von 15 mg/kg Morphin und 1·0 mg/kg Laevallorphan Sauerstoffverbrauch, Rektaltemperatur, ATP-, ADP- und Glykogengehalt des Gehirns keine signifikanten Abweichungen von der Norm. Die Spontanmotilität war gering gesteigert, der Kreatinphosphatgehalt des Gehirns noch um 0·30 µM/g verringert. Der durch Morphin herabgesetzte Milchsäuregehalt des Gehirns wurde durch Laeval-

lorphan nicht beeinflußt. Fünf mg/kg Laevallorphan normalisierten die durch Morphin veränderte Spontanmotilität, die Rektaltemperatur und den Kreatinphosphatgehalt des Gehirns. Der Sauerstoffverbrauch war signifikant gesenkt, desgleichen der ATP-, ADP- und Milchsäuregehalt des Gehirns, der Glykogengehalt war erhöht.

217b The Influence of Morphine and Laevallorphan on Motility, Oxygen Consumption and Rectal Temperature and on the Creatine-Phosphate, ATP-, ADP-, Lactic Acid, Glycogen and Coenzyme-A Content of the Mouse Brain. C. J. Estler (Germany).

The effect of morphine and Laevallorphan (3-hydroxy-N-allylmorphinan) when administered either separately or in combination on spontaneous motility, oxygen consumption, rectal temperature and the creatine-phosphate, ATP, ADP, glycogen, lactic acid and coenzyme-A content of brain was investigated 1 hour after they had been given to white mice. Laevallorphan in doses of 0·5, 1·0 and 5·0 mg/kg s.c. did not cause any significant changes in the metabolic processes tested or in motility. Fifteen mg/kg of s.c. morphine caused an increase of spontaneous motility to 18 times the normal as well as a decline in oxygen consumption by 12 per cent and a lowering of the rectal temperature by 2·2 °C. In the brain, morphine caused a rise in the ATP-level from 1·94 to 2·17 μM/g; the ADP-level fell approximately by the same amount; the creatine-phosphate level fell from 3·24 to 2·67 μM/g, the lactic acid level from 1·49 to 1·19 μM/g. The glycogen and coenzyme-A content remained unchanged. Laevallorphan in doses of 1·0 mg/kg or to a lesser extent of 0·5 mg/kg had a morphine-antagonistic effect. For example, 1 hour after the simultaneous administration of 15 mg/kg of morphine and of 1·0 mg/kg of Laevallorphan the oxygen consumption, rectal temperature, ATP-, ADP- and glycogen-levels of the brain showed no significant changes from the norm. Spontaneous motility was slightly increased, and the creatine phosphate content of the brain was diminished by 0·30 μM/g. The lactic acid content of the brain which was diminished by morphine, was not affected by Laevallorphan. Five mg/kg of Laevallorphan counteracted the changes in spontaneous motility, rectal temperature and creatine-phosphate-content of the brain due to morphine. Oxygen-consumption was significantly diminished as well as the ATP-, ADP-, and lactic acid content of the brain; the glycogen content was raised.

218 Concerning the Analgesic and Antipyretic Activity of Some Anticoagulant Drugs and Vitamin K. V. Kovalčík (Czechoslovakia).

Siegmund *et al.* have described a new test on mice suitable for the investigation of weak analgesics (causing painful irritation in the viscera). Their method was used to study the analgesic properties of vitamin K, ethyl-biscoumacetate and phenylindandione derivatives. Although the analgesic properties of vitamin K are widely recognized, it was only possible to confirm this using d'Armour-Smith's method. In experiments where weak analgetics were found to be effective, none of the anticoagulants had any marked effects. However, analysing their effect on pyrexia it was found that some caused further increase of temperature, whereas vitamin K was practically ineffective. A critical analysis of the results was performed.

219 Analysis of the Central Effect of D-Tubocurarine Chloride in the Cat. H. Kumagai (Japan).

Cerebral electrical activities were recorded from six parts of the brain (caudate nucleus, amygdaloid nuclear complex, thalamic nucleus centre median, hippocampus, midbrain reticular formation, and cortex) of cats treated with gallamine triethiodide and kept under artificial respiration. About 30 min after microinjection of D-tubocurarine solution (0·006 ml of 3 mg/ml) into each of the subcortical nuclei, seizure discharges started in the hippocampus, then spread to other parts of the brain. Discharges were less marked when the cortex had partially been removed. Although similar seizure discharges were observed by topical application of the same drug to the cortex, the sequence of discharge spread was somewhat different from that after microinjection into other parts. The seizure discharges were not obtained by microinjection of analeptic agents which evoked seizure discharges when applied intravenously.

220 The Effect of Oral Antidiabetics on the Central Nervous System. A. K. Pfeifer, A. Káldor, É. Sátory and G. Pogátsa (Hungary).

In a previous paper it has been shown that Carbutamide (C) increases the toxicity and the narcosis potentiating effect of ethyl alcohol (Káldor, Pogátsa). As this effect is not correlated with hypoglycaemia, nor is the metabolism of ethyl alcohol influenced by C, a direct effect of the drug on the central nervous system was assumed.

In the present study it has been observed that C inhibits maximal electro-shock seizures in a dose of 100 mg/kg in rats, whereas Metrazol convulsions are unaffected. Spontaneous motility of mice remains unchanged, whereas locomotor hypermotility produced by Amphetamine decreases considerably. The compound decreasesAmphetamine toxicity, when the mice are kept in groups. When, however, each animal is kept separately, the toxicity remains unchanged. This observation should be emphasized, as tranquillizers exhibit similar characteristics, whereas hypnosedative drugs inhibit the toxicity in both circumstances.

Just as with ethyl alcohol, thiopental narcosis is also potentiated significantl The central effect of C is indicated by its analgesic characteristics, i.e.

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using the contact heat method the reaction time is prolongated.

The central nervous effect seems to be established, but only in doses which also have a hypoglycaemic effect. The degree of hypoglycaemia, however, is not in close correlation with the central nervous effect. Our results obtained with other sulphonamide derivatives (Tolbutamide, Chlorpropamide) and insulin differ in some respects from the results with Carbutamid. Thus, it may be assumed that the sedative effect is, at least partly, a special characteristic of these compounds.

221 Effects of Some Aza-cycloheptenyl Derivatives on the Central Nervous System. W. A. Behrendt and R. Deininger (Germany).

The actions of 1-phenyl-3-amino- [1′-aza-1′-cyclohepten-yl-(2′)] -5-oxy- [1′-aza-1′-cyclohepten-yl] -pyrazol (I); 1-phenyl-3-amino-5-oxy- [1′-aza-1′-cyclohepten-yl-(2′)]-pyrazol (II); 1-aza-cyclohepten-yl-2-methylaether (III) and *N*-[1′ aza-1′-cyclohepten-yl-(2′)]-phenylisopropylamine HCl (IV) on the CNS were compared by a series of pharmacological testing procedures. Compound I produced central nervous system depression (sedation and loss of righting reflex) whereas after administration of II, III and IV central nervous system stimulation (hyperactivity and hyperexcitability) was observed.

The conditioned avoidance escape response (rats) and the defense reaction (golden hamster) were suppressed by I. Duration of sleeping time produced by hexobarbital (mice) was strikingly prolonged by I. In rabbits an effect on the EEG could be demonstrated. I and II had no effect on metrazol-induced seizures in rats, III shortened the induction time, while IV acted as an anticonvulsive.

I and III were effective in protecting rats from electroshock-seizures. Only I antagonized the convulsant (tonic phase) and lethal effects of strychnine in mice. In cats the activity of the linguomandibular reflex and patellar reflex was abolished at equal doses by IV. II caused transient hyperexcitability of both reflexes. I diminished or abolished the linguomandibular reflex at doses, which did not affect the patellar reflex. III abolished the patellar reflex at lower doses than those required to block the linguomandibular reflex. I caused "head-drop" ($ED50$ 8·4 ± 0·92 mg/kg, rabbits, i.v.) without influencing nerve-muscle preparation (cats). The following LD_{50} values (oral administration) in mice were found: I = 4800 ± 190 mg/kg, II = 175 ± 9·88 mg/kg, III = 795 ± 48.4 mg/kg, IV = 398 ± 17·3 mg/kg.

Compound I was found to be the most interesting one out of the four derivatives. The mechanism of action is similar to mephenesin and other members of the central muscle-relaxant group.

222 The Effect of Some Central Analeptics on the Depressive Action of Reserpine and Chlorpromazine on Respiration. A. Nicák, M. Lukasiewicz and R. Špaldonová (Czechoslovakia).

The effect of bemegride, amiphenazole, pentazol, nikethamide and amphetamine on the depressive action of reserpine and chlorpromazine on respiration was studied experimentally in mice and rats.

These central analeptics were fipplied after a premedication with reserpine in three time-intervals: 5 min, 60 min and 240 min, and 60 min after a premedication with chlorpromazine.

The respiration was registered by means of a plethysmograph and the motility of animals was evaluated simultaneously.

The experiments show, that central analeptics do not antagonize the depressive action of high doses of reserpine and chlorpromazine on the respiration of the animals used in these experiments.

The predisposition to convulsions after a premedication with reserpine and chlorpromazine before the central analeptics is increased.

223 The Pharmacological Analysis of the Hyperthermia caused by Phenamine (Amphetamine). M. L. Belenky and M. Vitolina (U.S.S.R.).

The action of phenamine on the body temperature of cats and rabbits was investigated. During the experiments the animals were tied lying on their backs. Intraperitoneal injection of large doses of phenamine (15–20 mg/kg) caused a pronounced hyperthermic reaction in the course of which the temperature measured in the peritoneal cavity rose by 3–4°C. At the same time as the temperature in the peritoneal cavity began to rise, an increase in the skin temperature was also observed.

In thalamic cats the hyperthermic reaction caused by phenamine was somewhat more pronounced than in normal cats. This reaction was inconstant and more feeble in cats decerebrated at the level between the thalamus and the superior quadrigeminal bodies. If the decerebration was made at the level between the superior and inferior quadrigeminal bodies, phenamine did not cause any hyperthermic reaction.

Injection of hexonium or cyclamine in doses sufficient to block the autonomic ganglia did not prevent the hyperthermic reaction caused by phenamine. This reaction could be prevented or abolished by injection of procurane or diplacine in doses which produce muscular relaxation.

The hyperthermic reaction caused by phenamine is central in its origin and depends rather on an increase in heat production than on a decrease in heat loss. The development of this reaction is mediated by motor rather than autonomic nerve fibres. Apparently, the nerve–muscle junction

takes part in the transmission of impulses which are responsible for the central control of the metabolism in striated muscles.

224 Steroids and Tumour Growth in Wound Tissue. M. B. Williamson and H. B. Haley (U.S.A.).

It has been noted that when certain heterologous tumours (H.Ad.1 and H.Ep.3) were transplanted into adult female rats about 20 per cent of the growing tumours which were implanted into wounds were surrounded by a connective-tissue envelope. This reaction did not occur when the tumours were implanted into normal unwounded rats. Experiments were undertaken to attempt to increase the percentage of connective-tissue reactions. Since various steroid hormones are known to affect protein metabolism, especially during the healing of wounds, the effect of such stances on the tumour transplants was studied. Fourteen days after implantation of tumour-cell suspensions, the tumours in the control animals were somewhat larger than those implanted in wounds. Significant differences did not occur in all experiments. When cortisone or testosterone propionate was administered, the tumours were significantly larger in the control than in the wounded rats. That this was a local reaction was indicated by the fact that when tumour tissue was implanted away from the wound area in wounded rats the tumour grew to about the same size as in unwounded control rats. The effect of Nilevar (17-hydroxy-19 nor-17α-pregn-4-en-3-one), a non-androgenic anabolic steroid, was studied in wounded rats. It was found that the tumour growth was significantly greater when Nilevar was given to wounded rats than when the rats received none.

225 The Action of Cortisone in Promoting Heterologous Tumour Growth. G. A. H. Buttle and E. M. Kovacs (United Kingdom).

It has already been shown by Toolan and Handler that cortisone will promote the growth of certain human tumours transplanted in weanling rats and other animal hosts. Cortisone treatment will also promote the growth of methylcholanthrene-induced rat tumours transplanted into weanling rats. These tumours regress when the cortisone treatment ceases. It seems probable that the cortisone is suppressing a reaction between an antigen in the tumour and the lymphoid tissue or antibody producing system of the host.

An investigation has been made as to the nature of this antigen in the tumour tissue. Groups of weanling rats were injected with either suspensions of normal human tissue or with suspensions of human tumours a week before challenge with the transplantable tumour of human origin (HS1) and treatment with cortisone. The antigenic potency of these tissues in producing antibodies against the tumour growth has thus been compared. It has been found that the transplantable tumour HS1 is the best antigen and that it is equalled in potency by some fast growing tumours removed from patients, notably melanotic sarcomata and sarcomata of bone; embryonic human spleen is just as efficient as tumour tissue, and embryonic human muscle is slightly less potent. Weanling rats injected with suspensions of these tissues are completely or partially resistant to subsequent tumour implantation. Slower growing tumours like carcinomata of the stomach and breast, on the other hand, are ineffective as antigens and the subsequent HS1 tumour implants grow well. Muscle from adult patients is less effective as an antigen than embryonic muscle. In the case of other tissues, human red cells have a very slight effect, but plasma and white cells are ineffective.

The experiments indicate that the antigen in the transplantable human tumour HS1, the response to which is suppressed by cortisone, is similar to antigens found in fast growing human tumours and also in human embryonic tissue.

226 The Effect of Living Haemolytic Streptococci on Ascites Sarcoma 37 and Krebs 2 Carcinoma Cells. H. F. Havas and J. J. Freed (U.S.A.).

In previous studies toxins from *Streptococcus pyogenes* and *Serratia marcescens* were found to cause regression of well established transplanted mouse tumours. Streptococcal preparations enhanced the oncolytic effect of Serratia preparations and lowered their toxicity but were less effective when used alone. Experiments were therefore undertaken to establish if living streptococci could destroy the neoplastic cells. The effects produced by *S. pyogenes* strains were compared with the activity of *S. marcescens*, *Sarcina lutea* and *Streptococcus faecalis* by *in vivo* and *in vitro* studies. The resting bacterial cell suspensions were incubated for 1½ hr with ascites Sarcoma 37 and Krebs 2 carcinoma cells. These suspensions were then treated with antibiotics and injected into groups of 50 ICR Swiss mice totalling 2400. Three out of 8 streptococcal strains were highly effective against the tumour cells (0–30 per cent takes with treated tumour cells; 10 per cent takes in the controls). *Sarcina lutea*, *S. marcescens* and *S. faecalis* were only partially effective. Data on weight changes, tumour sizes and takes, and survival rates will be presented.

The absence of immunity in animals having previously received streptococci treated tumour cells indicated that exposure of these cells to streptococci altered their antigenic configuration.

The effects of the bacteria on tumour cells were studied at the cellular level with phase microscopy. The streptococci produced cytopathogenic effects such as blebbing, swelling and pynocytosis which soon led to the death of the tumour cells as recorded in a time lapse film.

227 Actual and Potential Influence of Mutagens on Trypanocidal Activity. N. Ercoli and R. Martinez (Venezuela).

Repeated exposure of *T. equiperdum* to nitrogen mustard *in vivo*, through successive passages in mice, formed a strain which required higher tartar emetic doses for clearing, while the curative action—established by the classical reinoculation method—decreased. The paradoxical drug behaviour was clarified by the demonstration that nitrogen mustard treatment changed the immunological properties of the trypanosomes to the point that the original strain used for reinoculation behaved as a heterologous one, inducing positive infection in non-cured mice also.

With other drugs, nitrogen mustard also modified the spectrum of drug sensitivity; activity was increased by mustard in the case of stilbamidine and acriflavine, decreased in the case of phenarsone and carbarsone. In the case of tartar emetic which cannot produce resistance *per se*, the immediate clearing action took place, but the delayed effect was markedly decreased or abolished.

The formation of complete resistance to acriflavine in the nitrogen mustard-treated trypanosomes was rapid, being obtained after six passages. The acriflavine-fast organism became completely tartar emetic-resistant after a single treatment.

These findings suggest that an unforeseeable number of variations in the reactivity of protozoa to chemical agents may be expected following exposure to mutagen doses which do not otherwise interfere with the reproductive rate of the parasite.

228 The Effect of Human Catalytic Enzymes upon the Duration and Distribution of Antimetabolite Action: the Guanase 8-Azaguanine System. T. C. Hall, R. Levine and C. A. Harris (U.S.A.).

The therapeutic effectiveness and organic toxicity of 8-azaguanine (8 AG) has been found to be related to the concentration of the catalytic enzyme guanase in body tissues. Spectrophotometric measurement of guanase levels in frozen human normal and tumour tissues from 37 patients has shown that tissues can be grouped according to enzyme activity into a "high" group, including brain, liver, small intestine and kidney with levels of 300–2500 μ/mg wet/wt; an intermediate group, including stomach, pancreas and spleen with levels of 80–200 μ; and a low group, with levels of 0–60 μ: spleen, gingiva, lymph nodes, adrenal, heart and cancer tissues. Eleven of 14 miscellaneous malignancies fell into the low group. The toxicity of 8 AG to various body tissues is strictly related to the level of the enzyme present, as is its therapeutic effect against tumours.

Oral administration of 8 AG is ineffective; this can be related to the high level of catalytic enzyme in small intestine which converts 8 AG into inactive 8-azaxanthine. Intravenous injection of 8 AG results in skin rashes at high levels; moderate levels of 8 AG are rapidly destroyed by liver guanase. The rate of destruction is rapid and quantitative conversion to 8-azaxanthine occurs within 24 hr.

Prolongation of the action of the drug on tumour tissue can be obtained if it is infused slowly through the arterial supply to sites of tumour involvement. Slow intra-arterial drips can be given and result in regressions in tumours with low guanase levels.

229 Pharmacology of Carcinolysis. R. Willheim, P. Fluss and B. B. O'Malley (U.S.A.).

In an attempt to elucidate the chemical basis of the phenomenon of serum carcinolysis, Willheim turned to results of O'Malley and Osawa who demonstrated pharmacological antagonism in terms of the cytolytic and growth depressant effects of RNase and the inhibition of cytolysis by adenosine-5-monophosphate (A5MP) and other nucleotides in a variety of cell systems.

It was found that not only did RNase and certain psychopharmacological agents markedly increase serum carcinolysis, but further, that A5MP and other nucleotides inhibited carcinolysis of ascites tumour cells *in vitro*. Cytochemical evidence using the Pappenheim technique indicated that carbinolytic agents effected a destruction of DNA and RNA of the tumour cell.

In vivo studies demonstrated that Compazine in particular, as well as fatty acids, niacin and antipyrine markedly inhibited ascites tumours when administered intra-peritoneally and subcutaneously. Moreover, extended time studies pointed to an immune basis for the action of carcinolytic agents, since the therapeutic index increased as the time for initiation of therapy was extended from the date of innoculum. Cortisone and X-radiated studies confirmed this finding in the case of Compazine.

Ascites tumour cells incubated with carcinolytic agents were found to undergo a loss in their carcinogenic potency.

230 Cytostatic Properties of *Opunta Maxima*. J. W. H. Hovy (South Africa).

Aqueous extracts of dried *Opunta maxima* (spineless cactus) orally administered extended the latent period of neoplastic growths in female mice. A single i.m. injection of powdered material in oily suspension more clearly augmented this retardation and prolonged the life of test animals. Repeated subcutaneous infusions of concentrated aqueous solutions, however, potentiated the carcinogenic effect of methylcholantrene, reducing the latent period and increasing the growth rate of tumours, but no carcinogenic properties of the substance per se could be detected. In males, 0·25 mg methylcholanthrene developed tumours after a much longer latent period, while the rate of tumour growth was slower than in the females. Overall reaction to *Opuntia maxima* treatment was not different in both sexes, but lower susceptibility to methylcholanthrene in males was concomitant with

potentiation of the methylcholanthrene by the plant powder in doses causing retardation of growths in females. The suppressive effect was inversely proportional to the dose. The same effects were obtained with injections of benzol, ether, alcohol and acetone extracts in olive oil. Methylcholanthrene affects fertility adversely in the male and not in the female; *Opunta maxima* orally administered affects female fertility adversely and does not appreciably influenced reproductive capacities in the male.

The active ingredient is slight soluble in water and highly soluble in lipoid solvents. Optimum suppressive effect was obtained with greatly diluted oily solutions of the highly potent ether extract administered 2 weeks after methylcholanthrene injection. Oily suspensions of powder gave complete protection in some cases surviving for more than one year. A possible mechanism of substrate competition is discussed.

231 Cure of Spontaneous Cancers with Antimetabolites Directed Against the Synthesis of Vitamin B_{12}. D. W. Woolley and J. M. Stewart (U.S.A.).

Earlier work from this laboratory had shown that spontaneous mammary cancers of Swiss mice could be caused to regress or to disappear temporarily by treatment with antimetabolites of 1:2-dimethyl-4:5-diaminobenzene (DMDA). These antimetabolites inhibit the biosynthesis of vitamin B_{12}. They harm the cancer, but not the host animal because the cancer synthesizes this vitamin, whilst the host does not. Two shortcomings of these early antimetabolites were: (a) they affected only some of the cancers in a group of mice; and (b) they did not cause permanent destruction of the cancers, only temporary. Efforts to discover better antimetabolites of this class have continued, with the result that several have been found which cause permanent cures of some cancers. Three of these new compounds related structurally to DMDA caused permanent cures of the cancers of half the mice. In these spontaneous cancers, no beneficial effect can be shown for any of the antimetabolites such as aminopterin or fluorouracil which inhibit some transplanted cancers. The present compounds are thus the first known to be able to cure spontaneous cancers. Their discovery was the result of an understanding of the metabolic differences between cancers and normal tissues.

232 An Effect of 1-Mercapto-2-aminobutane (MAB) on the Anti-Tumour Activity of Nitrogen Mustards. R. J. Rutman, F. S. Lewis and C. C. Price (U.S.A.).

The mercapto-alkyl-amines are known to reduce the lethality of both X-rays and nitrogen mustards. We have now studied the effect of this protective action on HN_2 treatment of ascitic form lymphosarcoma and leukaemia in mice. MAB reduces the toxicity of HN_2 by 50 per cent; despite this the chemotherapeutic activity against the lymphosarcoma is not altered. In contrast, when the acutely leukaemic L1210-bearing host is pre-treated subcutaneously with MAB, HN_2 intraperitoneally administered thereafter shows an increased chemotherapeutic index ($^{MTD\text{-}MED.}/_{MED}$), significantly greater effects on survival time (controls—7·0 days; HN_2 alone—8·0–9·0 days; HN_2 + MAB—14·0 days) and on the number of survivors at 150 and 200 per cent of the control survival time. MAB was more effective than its lower homologue, 1-mercapto-2-aminoethane. Whereas protection cannot be obtained if MAB is administered over 15 min after HN_2, the reverse situation obtains for L-phenylalanine mustard (CB3025), which is not detoxified by prior administration of MAB but is counteracted after a 24-hr delay. Extension of the anti-tumour activity of CB3025 also requires delayed injection of MAB. Incidental observations suggest that MAB activity is influenced by such factors as ambient temperature and by the occurrence of hyperactivity in the experimental animals. The differential effects on host and tumour reported above suggest protective action is more subtle than direct inactivation by MAB of the administered alkylating agent.

233 The Significance of Sarcomas Induced in Rats and Mice by Iron-Dextran. L. Golberg, J. P. Smith and S. B. de C. Baker (United Kingdom).

An assessment of the risk of neoplasia incurred by injecting iron-dextran in man must be based on an understanding of the pathogenesis of the tumours in rats and mice, a comparison with the injection site in man and delineation of the relationship between the experimental conditions in animals and the circumstances of clinical use of iron-dextran.

Repeated injections of 0·2 ml iron-dextran (10 mg Fe) subcutaneously into the same site in the mouse elicit irreversible fibrosis progressing to foci of fibroblastic proliferation (considered to be pre-neoplastic) only if 6 or more such injections are given. Up to 10 injections of 0·02 ml iron-dextran are without any effect of this kind even after a year. Similar experiments were carried out in rats given 0·5 or 0·05 ml iron-dextran. These results, taken in conjunction with quantitative measurements of iron absorption from the injection site in various species, lead to the conclusion that the effect of a given dose must be related to the bulk of tissue into which it is injected.

A series of experiments on the connection between dose of iron-dextran and tumour yield in mice and rats strongly suggests a dose–response relationship, dose being calculated on a basis of body wt. Finally, histological examination of sections from gluteal injection sites in patients who had received clinical courses of iron-dextran up to 3 years earlier did not reveal changes resembling in any way the pre-neoplastic effects mentioned above. Thus, these

further investigations make it clear that the risk of neoplasia in man is remote.

234 Relationship between Water Permeability and Integrity of Sulphydryl Groups in Malignant and Normal Cells. M. Belkin and W. G. Hardy (U.S.A.).

When malignant cells, animal and human, were exposed *in vitro* to solutions of heavy metals or other selected compounds, three types of cell blebs were produced: (1) acentric blebs arising from one side of the cell, e.g. by chlormerodrin, meralluride sodium, mercuric chloride; (2) symmetrical blebs which completely enveloped the cell, e.g. by strong silver protein, auric chloride, *p*-chloromercuribenzoate; and (3) scallop blebs, numerous small spherical elevations which completely covered the cell, e.g. by *N*-ethyl-maleimide, trivalent arsenicals, iodoacetamide. As indicated by vital stains and morphologic appearance, the blebs arose in healthy cells. They also can be made to appear *in vivo* in ascites tumour cells by intraperitoneal administration of an appropriate blebbing agent. All the bleb-producing chemicals have the property of reacting with protein–sulphydryl groups by alkylation, oxidation or mercaptide formation.

The three bleb types have been induced in 8 mouse and 2 rat ascites tumour cells, and in 4 human and one mouse malignant cell line and 3 normal cell lines grown in tissue culture. In contrast, cells from normal solid tissues of liver, lung, spleen, kidney, testis and brain from mouse, rat, and rabbit failed to produce blebs. A possible interpretation for these observations will be presented.

235 Analysis of the Growth-inhibitory Effects of Hydrocortisone on a Mouse Lymphoma Cell growing in vitro. L. Aronow and J. D. Gabourel (U.S.A.).

Mouse lymphoma P-388, growing *in vitro* in Eagle's medium supplemented with serine, pyruvate, and calf serum (5 per cent), has been shown to be highly sensitive to hydrocortisone (F) and certain other natural and synthetic steroids of high anti-inflammatory potency. Several other steroids (11-epihydrocortisone, 11-keto steroids, sex steroids and cholesterol) had little or no effect on the growth of these cells.

The effect was studied further by analyzing the cellular content of protein, ribonucleic acid, deoxyribonucleic acid, and lipid in cultures with and without added F. Glucose utilization was also measured. Cells treated with 5×10^{-6} M F showed a decreased rate of glucose consumption and a decreased rate of protein accumulation within the first 24 hr. Experiments with radioactive leucine confirmed the suspicion that an early effect of the steroid was a decreased rate of protein synthesis.

Continued culture of these cells in sub-lethal concentrations of F led to the isolation, after 4 weeks, of an F-resistant sub-line. The resistance is maintained in cells cultured for long periods of time in the absence of added steroid.

The ID_{50} (concentration of F necessary for 50 per cent inhibition of normal growth) of the sensitive cell line is about 7×10^{-6} M; the ID_{50} of the resistant cell line is greater than 10^{-4} M. Cross-resistance extends to all the potent anti-inflammatory steroids tested, both natural and synthetic.

236 Nicotine Inhibition of Normal Cell Growth through Lowered Oxidation States. M. M. Brooks (U.S.A.).

Pure solutions of nicotine in concentrations of ·00001 per cent to ·000003 per cent in sea water produce a change in the form of larva at the beginning of the gastrula stage of two different sea urchins, namely, *Strongylocentrotus purpuratus* from the California coast, and *Lytichinus atlanticus* from Bermuda. This shape known as exogastrula, has been found by others using certain chemically unrelated reagents, which have been classified by the writer as "reducing reagents". They change the normal ratio of oxidants to reductants of the enzyme systems in the scale of metabolism to a more negative redox potential whereby the reductants predominate. This changes the ratio to abnormal values in each successive step. The final step, that of the activation of oxygen, is thereby limited in producing the essential energy for further growth. During growth, changes in potential for the production of the various structures occur. When the normal balance of oxidants to reductants at the various stages of metabolism is upset, the organism cannot grow to maturity. This is the case at the gastrula stage when a block occurs, so that the organism remains in the immature state, producing several globular parts extruding from the base instead of invaginating. Since nicotine produces the same embryonic form, it can also be classified as a reducing reagent, producing a more negative redox potential and lowering the energy released which is necessary for further structural development. These experiments are based on the theory of lowered energy levels through enzyme inhibitions as a cause of cancer and as illustrated by experiments with nicotine.

237 The Concept of Quantitative Biochemical Differences between Tumour and Host as an Approach to Cancer Chemotherapy. L. S. Dietrich and D. S. Martin (U.S.A.).

The niacin antagonist 6-aminonicotinamide (6-AN) causes a reduction of tumour size in the 755 adenocarcinoma grown in C57 mice.[1] Administration of 6-AN results in an inactive pyridine nucleotide (PN) analogue containing 6-AN and markedly inhibits the tissue activity of various PN-dependent enzymes, in particular those associated with aerobic and anaerobic high energy phosphate synthesis.[2] Employing β-hydroxybutyrate oxidase (BDO), a PN-dependent system as a model,

the level of drug needed to produce an inhibition in a given tissue has been correlated with the BDO activity, the total PN and PN analogues levels of the tissue. Seven tissues were studied and found to have BDO activity in the following order of decreasing activity: liver, kidney, brain, heart, 755 tumour, lung and skeletal muscle. After excluding skeletal and cardiac muscle, which are unable to incorporate efficiently 6-AN, the other tissues studied fall into a pattern. At low level of 6-AN therapy, tissues with low BDO activity and PN concentrations such as the 755 tumour exhibited antagonism of BDO activity. Tissues such as liver, which have higher PN levels and enzymatic activities require higher levels of 6-AN before enzymatic alterations are observed.

The significance of these studies will be discussed in relationship to the utilization of quantitative biochemical differences in the selective destruction of cell types.

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1. (1957), *Cancer Res.*, **17**, 600.
2. (1958), *J. Biol. Chem.*, **233**, 964.

238 Inhibitory Action of Drugs Against Intracerebral Leukaemia. A. Goldin and M. Chirigos (U.S.A.).

The clinical importance of intracerebral (I.C.) leukaemia prompted chemotherapeutic studies of this syndrome in mice. Model systems were employed, utilizing intracerebrally inoculated or infiltrated leukaemic cells. Dose-response studies involving drug administration at intervals preceding I.C. inoculation of leukaemic cells determined the time-course of effective levels of drug in the brain. Administration of drug after I.C. inoculation coupled with transplantability-bioassay of tissues at intervals and survival time determination in treated and recipient mice served to measure extent and duration of drug action.

*Cyclo*phosphamide (Cytoxan) on various schedules was significantly less effective in extending survival time of mice with I.C. as compared with subcutaneous leukaemia. This was attributable to reduced penetration of the drug into the brain, and the more favourable site for leukaemic growth afforded by brain. Bioassay indicated that Cytoxan inhibition of leukaemic cells was diminished in extent and duration for brain as compared with peripheral blood or other tissues. Evidence was obtained that, following injection, Cytoxan or an active metabolic product was present in effective levels for approximately one hour in blood and tissues, and for a lesser period in the brain. Similar methodology was employed in comparative studies of the therapeutic efficacy against I.C. leukaemia of a series of chemotherapeutic agents, including alkylating agents and folic acid and purine antagonists. The data indicate that the sequestration of leukaemic cells in the brain and inefficient entry of drug to the brain site may contribute to a refractory state in therapy.

239 The Passage of a Large Toxic Molecule (Botulinum Toxin) Through Living Membranes. G. J. Hildebrand, R. J. Heckly and C. Lamanna (U.S.A.).

The permeability of various living membranes for a large toxic protein molecule and the mode of permeation was studied by determining the distribution and the size of the toxic unit of crystalline botulinum toxin type A (mol. wt. 900,000) in various body fluids after intraduodenal and intravenous instillation.

Only a small fraction of intraintestinally instilled toxin passed the intestinal barrier in the rat and rabbit and this is limited to passage into lymph. Ultracentrifugal analysis yields $S_{20}w$ values for the absorbed toxin which are considerably lower than those for the instilled toxin.

After intravenous instillation in rabbits, no toxin passed across the blood-spinal fluid barrier or across the placenta. Small quantities appeared in bile, urine and intraocular fluid. In lymph, toxicities were found ranging between 1/6–1/12 of the plasma toxicity. The $S_{20}w$ values of the toxic unit in plasma and lymph were about 50 per cent lower than those of the injected toxin.

Measures which would increase the filtration rate through the capillary wall, lead to a greatly increased toxicity of the body fluid. This together with the high toxicity of lymph is taken to indicate that the passage of the toxic unit across the capillary wall is effected predominantly by filtration mechanisms. The findings indicate that the toxic unit of botulinum toxin A, which passes through living membranes and eventually reaches the receptor site is considerably smaller than the original 900,000 mol. wt. toxic molecule, but still lies within the range of plasma proteins. Part of the decrease in size is attributable to dissociation of a protein hemagglutinin from the neurotoxic protein in crystalline toxine.

240 Increased Resistance to Bacterial Toxins Induced by Phenol. J. Vaněček and H. Rašková (Czechoslovakia).

Experiments are presented which confirm the previous finding that repeated injections of small doses of phenol increase the resistance of experimental animals to various toxins.

It was found that repeated phenol injections lead to a stimulation of a factor in the blood which increases the resistance to toxin. Furthermore, the increase of resistance which is a temporary phenomenon is transmitted from the phenolized mother to her young. The transmission is at least partly caused by a factor in the milk as it was possible to stimulate the resistance of non-phenol treated young to toxin, if they were fed from birth by mothers who had been repeatedly given phenol injections. The observed phenomena are discussed.

241 The Influence of Body Temperature and Chlorpromazine on Tetanus Toxin Intoxication. J. Elis and I. Janku (Czechoslovakia).

The treatment of tetanus is still a great problem. Recently, chlorpromazine has been widely used with conflicting results. Chlorpromazine lowers the body temperature. Lowering of body temperature by itself improves the course of tetanic experimental intoxication (Weiss, Wright). An attempt was made to study the effect of chlorpromazine and lower body temperature in relation to the course of tetanus toxin intoxication. The experiments were performed on mice. Intramuscular and intravenous administration of tetanus toxin were used. Groups of animals were kept at laboratory temperature of 8°C. Chlorpromazine was injected in doses of 0·75 mg/kg i.m. three times a day. The first injection was given after the tetanus toxin administration. Temperature reading was performed twice a day rectally. A factorial analysis of the results was performed. Low room temperature as well as chlorpromazine prolong the mean survival time of the animals. Both factors applied together further increase the survival time. The mechanism of the observed phenomena are discussed.

242 The Protective Effect of a Serotonin Antagonist on the Lethal Toxicity of a Bacterial Toxin, Streptolysin O. S. P. Halbert, R. Bircher and E. Dahle (U.S.A.).

Highly purified preparations of streptolysin O from a group A or a group C streptococcal strain have been shown to cause very rapid death in rabbits and mice upon intravenous injection. It was demonstrated that profound electrographic changes were invariably produced prior to the clonic and tonic convulsions which preceded death. With 3–6 times the LD_{50} in rabbits, ventricular fibrillation or arrest could occur within as short a time as 3–5 sec, following the injection. Smaller doses usually caused death within 5 min, preceded by a variety of electrocardiographic disturbances. The electro-encephalogram was not apparently affected. Rabbits given lethal doses revealed strikingly elevated serum K levels at the time of death. These were usually associated with variable amounts of intravascular haemolysis.

It was found that a serotonin antagonist, l-methyl-D-lysergic acid butanolamide bimaleate (methysergide) significantly prolonged life when given to rabbits prior to the challenge dose of streptolysin. In a large number of experiments in mice, pretreatment with methysergide caused a small percentage of animals to survive one LD_{100} in addition to a prolongation of life in those which succumbed. Other drugs, including parasympathetic inhibitors and stimulants, sympathetic inhibitors and stimulants, Na salicylate, Ca ions, and a chelating agent failed to reveal any significant effect on streptolysin toxicity. Some other serotonin antagonists revealed less pronounced protection.

243 Release of Pharmacologically Active Substances by Staphylococcal Toxin (Wood 46). B. Wiegerhausen and H. Raškova (Germany).

The effect of staphylococcal toxin (S) has been studied *in vitro* on the organs of various species. S was prepared as a filtrate and stored at about −10°C. The toxicity of S (LD_{50}) in albino mice has scarcely changed in 6 months.

S causes effects according to the dose applied to the smooth muscle organs. There are characterized by:

(1) A latent period between the application of the toxin and the beginning of the reaction. Responses take place after a short latent period in isolated strips of rat stomach, duodenum, ileum and colon, guinea-pig ileum and chicken caecum, but after a longer period in guinea-pig and rat uterus.
(2) A slow increase in tonus. The muscle contractions proceeds to spontaneous motility.
(3) A maximal response of submaximal contraction of varying length of duration.
(4) A change in responsiveness of the organ to repeated administration of toxin after the end of reaction.

The response to small doses can be washed out and is reversible, whilst there is no effect at high concentration on the heart of *Helix pomatia*. The blocking by LSD and other serotonin antagonists of the S effect on rat histamine-insensitive uterus and the stopping by atropine of the spontaneous motility produced by S in guinea-pig ileum indicate a serotonin-like mechanism. Since an increase in the tonus of smooth muscle organs is not associated with these antagonists, another muscle stimulant is presumably responsible.

On the basis of our experiments, the assumption of a direct muscle stimulating effect by S appears questionable. The release of a muscle stimulant by S is more likely.

244 Toxicological Studies on the Venom of a Sea Snake, *Laticauda semifasciata* (Reinwardt). Tsuchih Tu (Formosa).

The pharmacological properties of the venom of *Laticauda semifasciata* (Reinwardt) are as follows:

1. Toxicity.

		Subcutaneously (mg/kg)	Intravenously (mg/kg)
LD_{50}	mice	0·338 ± 0·013	0·211 ± 0·012
	guinea pigs	0·0897 ± 0·0028	0·0631 ± 0·0023
	rabbits	0·0495 ± 0·0027	0·0486 ± 0·0025
MLD	frogs	10–30	

2. It has a slightly stimulant action on earthworms and on the pig ascaris.
3. It produces a marked inhibition of respiration, and it causes a rise followed by fall in blood pressure.
4. It induces a stimulant action on the isolated frog heart, and a slight one on that of rabbits. The

coronary outflow of the rabbit heart sometimes show a slight increase.
5. It dilates the hind leg vessels of frogs in small doses, but in larger doses, after a transient dilation, constricts them. It constricts the ears vessels of rabbits after a transient dilation. It causes a construction of the visceral blood vessels and vena abdominalis of frogs.
6. The isolated smooth muscle of the rabbit intestine is stimulated by the venom in medium doses and first stimulated, then inhibited in large doses. The isolated smooth muscle of the rabbit uterus, the isolated earthworm muscle, and the isolated stomach and intestine of frogs are stimulated by the venom.
7. It has both a curare-like action and a direct paralytic action on the isolated frog gastrocnemius muscle.
8. It causes a rise of the blood sugar level of rabbits.
9. It has a haemolytic action on the rabbit erythrocyte.

245 On the Role of Calcium Ions in the Toxic Neomycin Action. M. Háva, V. Sobek and J. Mikulášková (Czechoslovakia).

The ability of basic antibiotics to influence the ionized calcium level has already been described in the literature. We observed a dependence of acute toxicity and chronic damage of the kidney and Corti-apparatus on the ionized calcium level after the application of neomycin to dogs, guinea-pigs and mice. The rise of ionized calcium was followed by decrease in acute toxicity, the lowering of ionized calcium by the opposite. The ionized calcium level was lowered by neomycin, while total calcium was unchanged and phosphorus was raised.

Vitamin D_2 whose administration leads to a permanently higher calcium level consequently abolished the nephrotoxic and ototoxic effects of prolonged administration of neomycin. This was histologically and histochemically proved.

The role of ionized calcium in relation to changes of potassium and sodium is discussed in the light of the results of these and further experiments.

246 Specificity and Mechanism of Development of Tolerance to Pulmonary Oedema Due to Inhalation of Irritant Gases. D. Henschler (Germany).

Inhalation of per se seemingly ineffective doses of respiratory irritants protects against subsequent exposure to multiples of the normal of LCt_{50}. This effect can be demonstrated with ozone, nitrogen dioxide, phosgene and chloropicrin, and each one of these irritants protects against the other. The protection does not become manifest before 12 hr following pre-exposure, it is optimal after 2–5 days and decreases after 10 days; a very small degree can be found up to 60 days. Repeated pre-exposure to equal doses at intervals of 2–4 days decreases the effect but continuous enhancing of the dose in serial exposures at 4-day intervals finally raises LCt_{50} to 6 times its normal value. Histological investigation of lungs in pregassed animals reveals minimal interalveolar oedema and slight septal inflammation the degree of which is parallel to the amount of tolerance. Protection against inhalation of irritants is also afforded by parenterally applied substances with lung oedema producing properties (thiourea); however, pregassing with irritants does not protect against oedema by thiourea or intravenous adrenaline. No significant pharmacological influence upon development of tolerance to irritants could be demonstrated. Alterations in rate or depth of breathing as the cause of protection could be ruled out. The nature of the phenomenon is therefore seen to be impeded diffusion of irritants from alveoli to capillaries by thickening of the alveolar wall. In animals pregassed with irritants impairment of diffusion could be shown with carbon monoxide (closed respirometry) or with nitrogen dioxide (methaemoglobin formation).

247 The Mechanism of the Retinotoxic Action of Sodium Glutamate. A. M. Potts and J. K. Freedman (U.S.A.).

It has been shown by others that administration of sodium glutamate to newborn mice causes destruction of the ganglion cell layer of the retina and failure of the bipolar cell layer to form. It was shown in our laboraoty that such animals have lost the b-wave of the electroretinogram. We have repeated these findings on the rat and have studied the metabolic activity of rat retina particularly in regard to glutamate metabolizing enzymes. We found complete loss of glutaminase I activity and retention of glutaminase II, glutamotransferase, and glutamosynthetase. These findings are consistent with our hypothesis that the presence of high concentrations of glutamate at the time of retinal development represses the normal formation of glutaminase I, causing irreparable damage to the layers wherein it fulfils an essential metabolic function.

248 Variation in Acute Toxicity of Sustained-release Amphetamine Preparations. A. B. Morrison, H. C. Grice and J. A. Campbell (Canada).

Female Wistar rats, averaging 100 g in weight, were given doses of D-amphetamine sulphate in standard gelatin capsules or sustained-release preparations. The doses were administered under light anaesthesia by the gastric insertion technique of Shenoy *et al*,[1] by making an incision in the greater curvature of the stomach, and placing the dose directly into the gastric lumen. Cumulative percentage deaths was plotted against time for the standard and sustained-release products, and median lethal times were calculated. Of 6 products tested at the level of 450 or 500 mg/kg of D-amphetamine sulphate, 4 showed evidence of sustained release, one was unavailable and one was similar to the

standard. Medium lethal times for the various products varied from 78 to 350 min. When the product which gave the same results as the standard at 500 mg/kg dosage level was compared with the standard at a lower dosage level of 200 mg/kg, however, it showed sustained-release properties as evidenced by delay in time of death. In further studies, the rate of absorption of amphetamine from the various products was determined by killing animals at intervals after dosing at the level of 100 mg/kg, and determining the amount of the dose remaining in the gastrointestinal tract. For 3 of the 5 products thus tested, the *in vivo* absorption data showed good agreement with toxicity data. It was concluded that sustained-release amphetamine preparations showed marked variability in acute toxicity.

1. Shenoy *et al.* (1960), *Toxicol. Appl. Pharm.*, **2**, 100.

249 The Influence of Biologic Disposition on the Sensitivity of Young Rats to Morphine. H. J. Kupferberg and E. L. Way (U.S.A.).

The intraperitoneal LD_{50} of morphine was determined in young rats between 1 to 32 days of age. The LD_{50} for 1 to 16-day-old animals was found to be between 35–60 mg/kg, but after 16 days it was found to rise sharply, the LD_{50} for 32-day-old animals being 220 mg/kg. The amount of morphine recovered in the whole animal for all groups 90 min after administration of 50 mg/kg intraperitoneally was approximately the same; likewise, no statistical difference was found in the amount of morphine conjugated. Blood levels of morphine in 16- and 32-day-old rats after 50 mg/kg i.p. were nearly identical; however, brain levels of 16-day-old animals were roughly two to three-fold those of 32-day-old animals. At equi-toxic doses—LD_{25} (50 mg/kg for 16-day-old; 150 mg/kg for 32-day-old)—the brain levels of both groups were approximately the same; however, the brain–blood ratios in the 32-day-old were generally about one-half those of the 16-day-old. The results suggest that the increased sensitivity of younger animals to morphine is due more to a lower blood–brain barrier rather than metabolic factors.

250 The Toxicity of Alkyl and Aryl Mercury Salts. J. C. Gage and A. A. B. Swan (United Kingdom).

Although the acute toxicities of alkyl and aryl mercury salts to rats are of the same order, their qualitative effects are quite different. Methyl mercury salts cause damage to the central nervous system which is manifested by a delayed muscular weakness and inco-ordination. No such toxic signs and no histopathological damage to the central nervous system can be produced with phenyl mercury acetate. The difference is more apparent after the repeated administration of small doses; no effects are produced with phenyl mercury acetate at five times the dosage rate which produces the characteristic effects with methyl mercury dicyandiamide.

In order to study the toxic action of these substances in more detail, analytical methods have been developed to determine organic and inorganic Hg in tissues and excreta. After subcutaneous administration to rats, phenyl mercury acetate is rapidly broken down to inorganic Hg and is excreted as such in the urine and faeces. Little is retained in the tissues apart from the kidney, from which it is excreted at a rate comparable to that observed after administration of inorganic Hg. No Hg accumulates in the central nervous system after repeated dosage. With methyl mercury dicyandiamide the breakdown to inorganic Hg is slight and it is only very slowly excreted, more appearing in the faeces than in urine. With repeated dosage, organic Hg is found in all tissues, and there is a marked accumulation in red blood cells and in hair. There is also a progressive increase of organic Hg in the central nervous system.

The results suggest that phenyl mercury salts are not more toxic than inorganic Hg, but the progressive accumulation of methyl mercury salts, and the severity and irreversibility of their neurotoxic action, renders them a much greater hazard. It seems unlikely that urinary determinations of Hg will be of value to control human exposure to these substances.

251 Some Non-linear Peculiarities in the Relationship between Fluoride Concentration and Biological Activity. A. Schatz and J. J. Martin (U.S.A.).

Inhibitions by toxic compounds usually increase with increasing concentration. There is a direct proportionality between dosage and effect until the curve levels off because of death or for some other reason, but there are well-authenticated and confirmed reports where toxicity at low concentrations is overcome or reversed simply by increasing the dosage of the same agent. If the concentration is then further increased, toxicity is again observed. Different authors have designated these unusual non-linear relationships between concentration and biological activity as "concentration quenching", "paradoxical effects", and "inversion phenomena".

This kind of peculiar effect has been observed with fluoride. The inhibition of human prostatic acid phosphatase by low levels of fluoride is partially reversed by higher concentrations. As the fluoride dosage injected into rabbits is increased, cholinesterase activity at first decreases, but then goes up. The autolytic release of phosphorus compounds from the alga *Scenedesmus Iuadricauda* progressively decreases but then rises as the fluoride concentration is increased. The appearance of dental caries is delayed by low levels of fluoride, but higher concentrations, paradoxically, increase tooth decay.

These peculiar, non-linear effects of fluoride occur under some conditions but not others. A straight line relationship does not always apply.

252 The Effect of Hydrazides on the Connective Tissue of Chick Embryos. D. J. Smith (U.S.A.).

It has been noted by Jenney *et al.*[1] that various hydrazides are potent convulsants in the mouse. We have now shown that many of these same compounds are capable of producing extensive tissue fragility and large amounts of soluble collagen when injected into 14-day chick embryos, similar to that noted by Levene *et al.*[2] for β-aminopropionitrile and semicarbazide in this system. Included in the list of such agents are thiosemicarbazide, carbohydrazide, thiocarbohydrazide, cyanoacetic hydrazide, isonicotinyl hydrazide, glycl hydrazide, γ-glutamyl hydrazide, and benzoyl hydrazide. Iproniazid (isonicotinyl-2-isopropyl hydrazine) and cyanoacetic acid do not cause these effects, suggesting that a free hydrazine group is necessary for activity in this system. The production of these lesions by aminoacetonitrile and β-aminopropionitrile would suggest an alternate requirement for activity of a uniquely active amino group. While pyridoxine and its derivatives have been previously noted as antidotes against the convulsant effects of hydrazides, they do not protect against the connective tissue effects of these agents in the chick embryo. In addition, penicillamine and cycloserine do not potentiate hydrazide activity, suggesting that vitamin B6 is not involved in the lesion.

(Supported in part by ONR contract Nour-3206(00).)

1. Jenney and Pfeiffer (1958), *J. Pharmacol.*, **122,** 110.
2. Levene and Gross (1959), *J. exp. Med.*, **110,** 771.

253 The Biochemistry of Methanol Poisoning. J. R. Cooper and M. M. Kini (U.S.A.).

From several lines of evidence it now appears that formaldehyde is the toxic agent in blindness due to methanol poisoning, and that the primary locus of action is on the retina. Using retinal slices and homogenates, it has been shown that pharmacological concentrations of formaldehyde produce a marked inhibition of glycolysis and oxidative phosphorylation, the latter system being the more sensitive. These findings support the hypothesis that retinal cell degeneration leading to blindness is the result of a deficiency of ATP. Whereas in liver mitochondria of cows and monkeys, formaldehyde at low concentrations is a substrate for oxidative phosphorylation, in retinal mitochondria of these species formaldehyde is a potent uncoupler of oxidative phosphorylation. Preliminary experiments indicate that the locus of action of formaldehyde on the phosphorylating pathway appears to be somewhere between flavoprotein and cytochrome c. Additional support for the thesis that ATP-generating systems are blocked in methanol poisoning comes from the finding that ^{32}P incorporation into phospholipids in retinal slices is markedly inhibited by formaldehyde.

Evidence will also be presented in favour of the role of alcohol dehydrogenase rather than the catalase-peroxide system being involved in the oxidation of methanol to formaldehyde.

254 Differentiation of Substance P from Other Polipeptides and Biogenic Amines on the Isolated Nerve Smooth Muscle Preparations. S. Huković (Yugoslavia).

Four different smooth muscle preparations with the nerve attached were prepared and set up in the isolated organ bath: (1) rat's; and (2) guinea-pig's ductus deferens with the hypogastric plexus, which is a postganglionic sympathetic nerve anatomically; (3) rat's urinary bladder; and (4) guinea-pig's rectum with the pelvic nerves. Both pelvic nerves are preganglionic, parasympathetic, anatomically. On electric stimulations of the nerves, good contractions of the smooth muscle could be recorded repeatedly.

Acetylcholine, histamine, 5-HT, aminobutyric acid, vasopressin oxytocin, bradykinin and substance P were investigated on the isolated organs.

Samples of substance P, had a distinct influence on the effect of nerves stimulations. Substance P increases the effect of the pelvic nerve stimulations of the guinea-pig's rectum and rat's urinary bladder and decreases the effect of hypogastric nerve stimulations of the rat's and guinea-pig's ductus deferens.

Other polypeptides and biogenic amines influence the effect of nerves stimulations differently.

255 Screening Drugs for Untoward Actions. B. P. McNamara, A. Pallotta, F. W. Oberst, J. Kodama, J. W. Crook, C. Graf and M. K. Christensen (U.S.A.).

The great increase in chemicals for drug and industrial use has necessitated assembly line methods to screen for obvious and subtle toxic actions. Ontoward drug actions are not always revealed by classical pharmacological techniques which measure changes in the cardiovascular, respiratory, neuromuscular, autonomic, or other body systems. Moreover, certain toxicological actions are revealed only by functional tests on unanaesthetized animals.

A small battery of tests has been employed with satisfactory results. These tests, which give a reflection of the mental and physical capability to perform, consist of: (1) Treadmill running in dogs; and (2) Conditioned avoidance responses in the dog or monkey.

This screening method is not specific as to the nature of the pharmacological action, it is designed to indicate that a given drug is likely to produce untoward reactions regardless of site and mechanism of action. Positive reactions have resulted from use of drugs which produced cardiovascular deficit (2-(1-naphthylamino)-imidazolene hydroiodide), impairment of heat regulation (Sernyl), tranquillization (chloropromazine), and central nervous system depression (pentobarbital and morphine).

In the development of the conditioned avoidance

tests it was found that the different behavioural responses varied in sensitivity to drug action.

256 The Effect of Certain Psychopharmacological Agents on the Amoeboid Migration of Human Leucocytes in vitro. M. KETCHEL and E. MILLER (U.S.A.).

The measurement of the distance that human leucocytes will migrate by active amoeboid motion has been utilized as an approach to the study of the mode of action of certain psychopharmacological agents.

Serotonin at a concentration of 1×10^{-6} μg/ml was found to have a pronounced inhibitory effect on the amoeboid migration of leucocytes. Increases in the concentration of serotonin between 1×10^{-6} μg/ml and 1×10^{-2} μg/ml caused increases in the inhibition of leucocyte migration. No further increase in inhibition was obtained when the serotonin concentration was raised from 1×10^{-2} μg/ml to 1 μg/ml. The inhibition caused by 0·01 μg/ml of serotonin was reversed by the addition of 0·01 μg/ml of chlorpromazine. Perphenazine and carbethoxysyringoyl methyl reserpate were ineffective in reversing the inhibition caused by 0·01 μg/ml of serotonin.

Lysergic acid diethylamide (LSD) at a concentration of 1×10^{-6} μg/ml was effective in inhibiting leucocyte migration. The highest concentration of LSD tested, 0·1 μg/ml, did not completely inhibit the migration of the leucocytes. Chlorpromazine at a concentration of 0·01 μg/ml was able to reverse the inhibition caused by 0·1 μg/ml of LSD. Partial reversal of the inhibition caused by 0·1 μg/ml of LSD was obtained by the addition of 0·1 μg/ml of perphenazine or 0·1 μg/ml of carbethoxysyringoyl methyl reserpate.

A combination of 0·01 μg/ml of serotonin and 0·1 μg/ml of LSD were not additive in causing inhibition, and the inhibitory effects caused by the combination could be reversed by the addition of 0·01 μg/ml of chlorpromazine.

257 The Estimation of 5-Hydroxytryptamine in Cerebral Tissue. D. D. BONNYCASTLE, M. F. BONNYCASTLE and E. G. ANDERSON (U.S.A.).

Bonnycastle *et al.* have demonstrated that treatment of rats with a variety of central nervous system depressant drugs (sedative, hypnotic, anticonvulsant anaesthetic and narcotic analgesic) result in significant increases over control levels, of brain 5-hydroxytryptamine. These determinations were made using acetone extracts of the brain assayed biologically on the clam heart (*Venus mercenaria*).

A comparison of the results obtained by this method of biological assay and those by the fluorometric procedure, as described by Bogdanski *et al.*, has been undertaken. An additional method of separation using ion-exchange columns has also been examined. The results obtained when these procedures of isolation and identification of 5-hydroxytryptamine have been applied to control animals and to animals treated with several representatives of the central nervous system depressant group of drugs will be presented.

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258 An Improved Method for Biological Determination of 5-hydroxytryptamine in Acetone Brain Extracts in the presence of Interfering Substances. Z. SUPEK and M. RANDIĆ (Yugoslavia).

While estimating the 5-hydroxytryptamine (5-HT) content of rat brain using atropinized rat uterus, it was observed that the acetone extracts contained substances which interfered with its biological determination. The interference could be due either to the inhibition of 5-HT contraction (catecholamines) or to the oxytocic activity of the interfering substance itself (cerebral factor of Andrews *et al.*). Attempting to eliminate this interference the following experiments were made. Dried acetone brain extract of 5 rats was dissolved in Jalon's solution and divided into two equal samples. One of these samples was enriched with a known amount of exogenous 5-HT (2 μg/g of fresh tissue). Both samples were tested on the same rat uterus using (2 and 2) dose assay as described by Schild in 1942. All assays with non-enriched samples proved to be invalid owing to significant non-parallelism of regression lines. The enriched samples showed very regular and valid assays with higher values of endogenous 5-HT. By adding artificially 5-HT to brain extract the enriched samples could be diluted to such an extent that all interfering substances are present in biological inactive concentrations. The limits of error ($P = 0{\cdot}05$) of assays of this type are relatively high, but on average did not exceed 20 per cent. This principle of eliminating the interference of biologically active substances in tissue extracts could be perhaps applied to bioassays of other substances.

259 A Simple Device for the Measuring of Fluorescence of Solutions by a Photographic Method. R. SMETANA (Czechoslovakia).

For many laboratories the lack of a spectrofluorometer considerably complicates research with catecholamines and other fluorescent compounds. Biological assays are much more expensive and time consuming. Therefore, we have made an attempt to work out a method, which would allow without great expenses a quantitative determination of these substances in very small amounts.

A device has been constructed on the following principle: The analysed sample is put into a cuvette, illuminated by light of a given wave length. The fluorescent light is filtered by a suitable filter, and directly transmitted onto a film. The time of exposure is determined empirically. The intensity of blackening of the film is in direct relation to the concentration of the fluorescent body and it is thus possible to make a quantitative determination by

densitometry. The concentration of the analysed compound is read from the calibration curve. Determinations of adrenaline and noradrenaline (from tissue extracts) by the trihydroxyindole method in comparison with biological assays are discussed.

260 A New Method of Registering the Ciliated Epithelial Motion in Experimental Animals. M. Mazur and A. Putowa (Poland).

The existing method of investigating the motion of ciliated epithelium (Kochman, Lomel) fails to register the changes which are due to the influence of drugs with expectorant or cough-allaying actions.

We have devised a method which allows the registration on a kymographical cylinder of the sliding of a piece of cork on the surface of the epithelium. The cork is linked with an electric system by means of a thread. The electric system consists of 2 platinum electrodes, direct current source (4 V accumulator), an electromagnetic writing device and a Deprez signal for checking the time. The cork when moving over a definite space cuts the current flow, the interval being registered by the pencil in the form of tracing on the cylinder. After the cork has slid over a definite space, the current is again switched on. This is marked by a second pencil tracing. By comparing the length of the segments on the graph in time units, it is possible to determine the motion speed of the ciliated epithelium being influenced by various factors. The experiments can be carried out on frog oesophagus epithelium and the tracheal epithelium of warm-blooded animals.

261 A Simple Biological Test for Oestrogenic Activity Employing Siamese Fighting Fish (Betta Splendens Regan). O. Ketusinh, N. Nilvibes and S. Banthudhiarm (Thailand).

The test depends upon a "grimace reaction" exhibited by the male Siamese fighting fish when opposed by another male member of the species. The procedure consists of adding a solution of the test substance to a measured volume (50 ml. or more) of Ringer's solution in which the fish has been placed. With sufficient concentration (about equivalent to 3 mg per cent of oestrone sulphate) a positive result—failure of the grimace reaction to develop—is obtained within one hour. The change, which is reversible, is distinct and practically unmistakable. Advantages of the method are simplicity, rapidity and economy with non-fatality and possibility of re-using test animals. Quantitative procedure may be developed for bioassay. One important limitation is that the test substance must be in aqueous solution.

262 A Contribution to the Methods of Testing Analgesics in Mice and Rats. M. Lukasiewicz, I. Ignácz and J. Chovan (Czechoslovakia).

A method for testing the analgesic action of drugs in mice and rats, is described.

Impulses of pain are produced automatically at regular time intervals. Motion reactions to these pain impulses are registrated by means of a kymograph; their number and intensity is summarized by means of a "collector of labour". The respiration is registrated simultaneously by means of a plethysmograph.

263 Application of the Technique of the Permanent Cannulation of Aorta and Vena Cava in Rats and Ground Squirrels (Film). V. Popovic and P. Popovic (U.S.A.).

A few days after operation, arterial and venous blood samples can be taken from the fully conscious animal. The insertion of cannulae takes about 8 min. The junction of both venae cavae is cannulated through the right jugular and the aorta is cannulated through the left carotid. Numbers 10, 20, 50 or 60 polyethlene tubes are used. The cannulated animal usually regains preoperational weight after 3 days. The life time of both cannulae reaches 40 days in rats (average 22 days) and 100 days in ground squirrels (average 47 days). The described technique has two major advantages: (a) it allows work on unanaesthetized and undisturbed animals; and (b) it allows simultaneous sampling and study of the characteristics of arterial and venous blood, e.g. the effect on the arterial blood when a drug or a hormone is infused into the venous stream.

After the demonstration of the insertion of both cannulae into the blood vessels, the film shows applications of the technique. In the second sequence, arterial and venous blood are withdrawn simultaneously from a rat running in a treadmill metabolism chamber. The third sequence of the film shows the rat exposed to low temperatures in a metabolism chamber. In this case the cannulae and extension tubes are electrically heated. In the next sequence the arterial blood pressure is measured in a freely-moving rat. The last part of the film shows an experiment on a ground squirrel. Venous oxygenation (A-V differences) and other blood characteristics are studied without interrupting hibernation.

264 Method for Simultaneous Numerical Computation of Different Types of Spontaneous Motility. W. A. Behrendt, R. Deininger and H. O. Hillers (Germany).

The method permits the simultaneous differential count of various types of motility in small laboratory animals. The equipment consists of a cage jointed and balanced by means of a torsion-spring. Numerical evaluation of the faculty of spontaneous motion is effected by means of a special photo-cell counter for registration of total movements and a contact-closing mechanism for counting locomotor activity. The physical and technical details of the apparatus are described. The effects of some centrally acting substances are discussed.

Thursday. August 24

265 Pharmacological Analysis of Central Nervous Action. Introductory Remarks. W. D. M. Paton (United Kingdom).

266 The Distribution of Possible Transmitter Substances. M. Vogt (United Kingdom).

There are a number of brain constituents of small mol. wt. which are so unevenly distributed in the central nervous system that their pattern of distribution alone suggests that they may serve a specialized function in those regions where they occur.

This function might, but need not, be that of chemical transmission of impulses. The following substances known to occur in certain parts of the brain and not in others will be discussed: Acetylcholine, histamine, the polypeptide "Substance P", the catecholamines (noradrenaline, adrenaline and dopamine), 5-hydroxytryptamine ("serotonin") and γ-aminobutyric acid.

Their distribution will be reviewed and the evidence for or against the theory that they act as transmitter substances discussed.

267 Substances Released in Nervous Activity. J. H. Gaddum (United Kingdom).

The theory of the chemical transmission of nervous impulses was originally based on experiments in which the active substances released on stimulation of the nerves were collected and identified. Direct evidence of this kind is more convincing than experiments on extracts, but more difficult to obtain, since the quantities are small. However, it was shown that the substance released in certain tissues was acetylcholine and not some other choline ester.

There have been various attempts to collect substances liberated in the central nervous system. They are apt to be destroyed locally, but they may diffuse into Ringers solution if this is brought in contact with the living tissue. Such solutions have been perfused for short periods through the dog's spinal cord[1] or allowed to run over the surface of the frog's spinal cord[2, 3] or kept in contact with the surface of the cerebrum in plastic cups[4] or run through push-pull cannulae, which continuously wash a small area of tissue.[5]

J. F. Mitchell, J. C. Szerb and I have been using the last two methods. Acetylcholine is liberated from the cerebrum of sheep and cats and the rate of liberation is increased by stimulating various nerves. At least one other substance has been detected by applying the solutions to goldfish intestine in a small bath (0·05 ml).

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268 Inhibitory Factors in the Brain. H. McLennan (Canada).

In recent years there have been a number of reports (e.g. those dealing with "Factor I") of the properties of extracts of the central nervous system which possess the ability to inhibit the activity of neurones and of some other excitable tissues. In the present paper the properties of such extracts will be discussed, and a comparison made of the actions of extracts prepared by various groups of workers. There are also many reports of the inhibitory actions of chemical compounds which are known to be present in the brain: the possible physiological significance of these, and also the extent to which the actions of the extracts can be ascribed to those of known compounds will be considered.

269 Discussion of the Previous Paper. Gamma-Aminobuturylcholine in the Central Nervous System. H. Kewitz (Germany).

270 The Effect of Drugs on Storage and Release of Serotonin and Catecholamines in Brain. E. Costa and B. B. Brodie (U.S.A.).

The storage of brain monoamines can be either increased or decreased by drugs affecting: (1) binding mechanisms of the monoamines; (2) specific enzyme systems controlling limiting steps for biosynthesis and metabolism; and (3) receptor sites controlling monoamine release. Each of these sites may be influenced by actions of drugs. The use of peripheral structures as models of events in brain allows a more detailed approach to the study of mechanisms. Drugs release monoamines from peripheral stores by: (1) non-competitive inhibition of binding mechanisms (reserpine, etc.); (2) mimicking the action of mediators which are responsible for their physiological release (guanethidine ?) and (3) competitive inhibition of binding mechanisms (tyramine, amphetamine, etc.). Selective affinity of drugs for different binding sites will be discussed.

Because of the limited knowledge of brain function one can only demonstrate concomitance between these biochemical changes and pharmacological effects in CNS. Thus α-methyl-metahydroxytyrosine (MMT) which selectively reduces brain catecholamines has been used as a tool to establish unequivocally that NE and dopamine depletion is unrelated to reserpine sedation whereas impairment of 5HT binding sites in brain is consistently associated with this pharmacological effect. An increase of free catecholamines in brain is associated with the excitatory state caused by reserpine in mice pre-

treated with a MAO inhibitor. The marked species differences in pharmacological and biochemical effects of reserpine and MMT reinforces the validity of these relationships. A mode of action of NE on neuronal excitability will be proposed using transmission of nerve impulses in sympathetic ganglia as a model. Here, free NE appears to serve as a modulator of transmission of impulses in sympathetic ganglia.

271 Interference with the Permeation of Aromatic Monoamines and Amino-Acids in Brain, a Possible New Type of Drug Action. A. Pletscher and K. F. Gey (Switzerland).

(1) Chlorpromazine and chlorprothixene in therapeutic doses counteract the following alteration of monoamine metabolism in rat brain *in vivo*: (a) increase of 5-hydroxytryptamine and 3-hydroxytyramine caused by inhibitors of monoamine oxidase like iproniazid, benzylhydrazine, isocarboxazid; (b) depletion of 5-hydroxytryptamine, norepinephrine and 3-hydroxytyramine due to reserpine and a benzoquinolizine derivative.

Imipramine and amitryptiline in comparable doses cause no similar alterations of cerebral monoamine metabolism.

Chlorpromazine does not inhibit oxidative deamination of monoamines, decarboxylation of aromatic amino-acids as well as the penetration of inhibitors of monoamine oxidase from the blood into the brain *in vivo*. Therefore, it may be concluded that chlorpromazine and probably also chlorprothixene inhibit the permeation of some monoamines to and from their storage organelles. This is supported by some reports of the literature.

(2) Chlorpromazine causes the following changes in the metabolism of aromatic amino-acids in rats: (a) diminution of the 5-hydroxytryptophan-induced rise of 5-hydroxytryptamine in brain *in vivo*; (b) inhibition of the transformation of 5-hydroxytryptophan to 5-hydroxytryptamine in isolated brain; (c) increase of the blood/brain quotient of ^{14}C-5-hydroxytryptophan or ^{14}C-3:4-dihydroxyphenylalanine.

Since the decarboxylation of aromatic amino-acids is not inhibited by chlorpromazine *in vivo* it can be concluded that the drug reduces the uptake of aromatic amino-acids by brain.

These results show that reduction of the permeation of monoamines and aromatic amino acids is a new possibility to interfere with monoamine metabolism.

272 Discussion of the Previous Paper. The Role of Dopamine in the Central Nervous System. A. Carlsson (Sweden) and G. M. Everett (U.S.A.).

273 Action of Cardiac Glycosides on Automaticity and Contractile Activity in Single Cardiac Muscle Cells Grown in vitro (film demonstration). A. Wollenberger and W. Halle (Germany).

Single, rhythmically contracting cells were grown in a sitting drop of a semisynthetic culture fluid from trypsin-treated ventricles of 13 day-old chick embryos. They were filmed, at 32 exposures/sec, after they had become attached to the surface of the cover slip. The isolated cells multiply on the glass and establish connexions, forming a synchronously pulsating reticulum and monolayer. In the monolayer cross-striated myofibrils become visible. The cyclic changes associated with contraction in the length of the sarcomeres and their component bands are for the first time witnessed in living cells. Addition of cardiac glycosides in concentrations of 10^{-9}–10^{-8} M strengthens the contractions and increases their frequency. In the case of digitoxin, these effects become maximal at about $1\text{–}2 \times 10^{-7}$ M, but at these and higher concentrations disturbances of rhythm and of co-ordinated contraction are noticed, such as irregular sequence of the pulsations, intra- and intercellular block of the propagation of the contraction wave, appearance of ectopic foci of activity (shown in single isolated cells), fibrillatory movements, and standstill. Much higher concentrations of structurally related glycosides without cardiac activity *in vivo* are ineffective. Most of the effects described are readily reversed by washing, as seen in an isolated cell placed in a perfusion chamber. The last scene shows a few cultured amnion cells, which on account of the low frequency and speed of their contraction permit a particularly impressive demonstration of the positive inotropic action of cardiac glycosides on pulsating cells.

274 Cardiac Glycosides and Ion Transport. J. B. Kahn, jr. (U.S.A.).

In cells which do work to maintain their ionic composition, metabolic energy is used to accumulate certain ions (e.g. K) and to exclude others (e.g. Na). Retention of electrochemical gradients represents a steady state of influx and efflux of ions. Cardiac glycosides affect many tissues (including the heart) from many species, by altering the steady states of cation influx and efflux. Unlike other compounds with similar effects on ion movements, glycosides have not been shown to have marked or consistent effects on energy-yielding metabolic processes. They seem instead to block active transport processes by interfering with the function of a carrier system responsible for uphill transfer of Na and K. In the heart, this effect can be related to the therapeutically useful positive inotropic effects of the drugs. Excitation in cardiac cells is associated with entry and exit of K and Na. Contraction is probably associated with changes in the state of internal Ca. From modern data on the mechanism of muscular contraction, on positive inotropic effects of glycosides, and on interactions of K and Ca in isolated mammalian hearts, a hypothesis can be

made to explain: (1) the coupling of excitation to contraction in the heart; and (2) the mechanism of the positive inotropic effects of cardiac glycosides.

275 Calcium and Digitalis Action on Heart Muscle. A. E. Farah and P. N. Witt (U.S.A.).

Changes in extracellular Ca concentration affect contractility of cardiac muscle. Ca acts on the excitable membrane, on the excitation-contraction coupling mechanism, and on contractile proteins. Digitalis effects are influenced by changes in Ca concentration. Thus an increase in Ca in the medium increases the toxicity of digitalis glycosides. The effects of digitalis on cardiac contractility are also related to Ca concentration, however, here the data indicate an additive effect of Ca and digitalis.

Ca content of the heart is not measurably changed by therapeutic doses of digitalis glycosides. Uptake of ^{45}Ca by the beating heart muscle is not changed by 10^{-7} M ouabain, while 10^{-6} M ouabain significantly increases uptake. The latter is a highly toxic concentration which causes contracture. Indirect evidence indicates that ^{45}Ca efflux is not significantly changed by therapeutic amounts of ouabain. Furthermore, there are distinct differences between Ca and digitalis actions on the heart. It is unlikely that digitalis in therapeutic doses acts through either increasing the rate of uptake or decreasing the rate of loss or changing the total amount of Ca in heart muscle. This does not exclude the possibility that intracellular redistribution of Ca may be operative, but this has not been studied.

Ouabain produces an increase in the rate-dependent inotropic effects of heart muscle. When the increase in contractility at high rates is depressed, ouabain in low concentrations (10^{-8} to 5×10^{-8} M) restores these phenomena. High or toxic concentrations of ouabain depress or eliminate the rate-dependent positive inotropic effects. It is proposed that therapeutic doses of digitalis act on the mechanism that causes the rate-dependent changes in contractility. Our results show that positive inotropic effects due to ouabain as well as Ca are not related to the changes in intracellular ionic composition as proposed by Hajdu.[1]

(Supported by a grant from the American Heart Association.)

1. Hajdu and Leonard (1959), *Pharmac. Rev.*

276 Cardiac Glycosides and Actomyosin. P. G. Waser (Switzerland).

For many reasons it is probable, that glycosides do not act only at the membrane phase of heart muscle but enter the sarcoplasma where they enhance the contraction mechanism. We have especially been interested in the action upon actomyosin. First the binding of Lanatoside A, C, or k-Strophanthin to actomyosin and myosin of skeletal muscle (rabbit and calf) and heart muscle (calf) was investigated. Ultrafiltration with a specially built apparatus and equilibrium dialysis gave the necessary data for the determination of the dissociation constants. The molar binding ratio was found to be larger for actomyosin than for serum-albumin.

The resulting glycoside–actomyosin complex changes the molecular form of the protein. The fibrous protein with high viscosity and double refraction of flow assumes an ellipsoidal shape after addition of glycoside to the solution (Ostwald viscosimeter). For many glycosides the change of viscosity is proportional to the biological property of fixation of the glycoside to the heart muscle.

The thixotropy of the actomyosin solutions was studied in a rotation viscosimeter. The coefficient of thixotropic breakdown obtained by application of constant shear shows an interesting correlation with the biological activity (toxic dose in the cat) of various active and inactive cardiac glycosides. This may be due to some charge phenomenon along the actomyosin fibre. Cardio-active glycosides were shown to augment binding of potassium and calcium ions to myosin solutions.

277 Relation of Cations to the Inotropic and Metabolic Actions of Cardiac Glycosides. K. S. Lee (U.S.A.).

The levels of ATP, ADP, AMP, PC and IP; the QO_2; the contractile tension and the Na^+, K^+ and Ca^{++} content of papillary muscles of cat were measured following the administration of ouabain to the medium containing varying amounts of K^+ and Ca^{++}.

(1) At inotropic stage of ouabain, the QO_2 increased whereas there was no change in either the content of the phosphate compounds or the issue content of Na^+, K^+ and Ca^{++}.

(2) At toxic stage of ouabain, the QO_2 was markedly increased while the PC and ATP content were decreased. In addition, the tissue content of K^+ was decreased whereas that of Na^+ and Ca^{++} was increased.

(3) Neither excess Ca^{++} nor K^+ lack in the medium produced the same effect on those parameters as that produced by ouabain.

The effect of ouabain on the glycerol extracted heart muscle fibre was also studied.

Dog heart muscle fibre extracted at —20°C in 50 per cent glycerol with 0·1 M histidine buffer at pH 7·00 for a short duration were found to contain a "relaxing factor". This "relaxing factor" was not present in fibres extracted or preincubated for a prolonged time.

Ouabain and Ca^{++} increased the ATP-induced contraction of these glycerol-extracted fibres containing the "relaxing factor". This effect of ouabain and Ca^{++} was not seen when the "relaxing factor" was absent from the fibres.

278 Metabolic Factors Controlling Duration of Drug Action. Introductory Remarks. B. B. Brodie (U.S.A.).

279 Detoxication Mechanisms in vivo. R. T. Williams (United Kingdom).

Most foreign organic compounds undergo some biochemical change in the body, although there are some pharmacologically important substances which appear to be biochemically inert. If these metabolic changes are considered from a pharmacological aspect, there appear to be five main types of reactions. From a chemical point of view, three of these do not involve synthesis but are oxidations, reductions or hydrolyses, whilst the other two are syntheses.

These reactions are: (1) those in which biologically inactive compounds are converted into active metabolites; (2) those in which biologically active compounds are converted into active metabolites with the same or different activity; (3) those in which biologically active compounds are converted into inactive metabolites by oxidation, reduction or hydrolysis; (4) detoxication mechanisms, i.e. syntheses between foreign compounds and body carbohydrates or amino-acids to yield inactive and non-toxic excretory products; and (5) lethal syntheses, i.e. syntheses between the foreign compound and a body constituent to produce an injurious product. Examples of these reactions are: (1) the conversion of chloral hydrate by reduction to the hypnotic trichloroethanol; (2) the conversion of codeine by oxidative demethylation to morphine; (3) the conversion of phenobarbitone by hydroxylation to the inactive hydroxyphenobarbitone; (4) the detoxication of phenol by conjugation with glucuronic acid; and (5) the synthesis of fluorocitrate from fluoroacetate and the consequent jamming of the tricarboxylic acid cycle.

The first three types of reactions are carried out mainly (but not entirely) by liver microsomal enzymes, i.e. the so-called microsomal "drug-metabolizing enzymes" and many of these have a requirement for reduced triphosphopyridine nucleotide and oxygen. The detoxication mechanisms require the participation of "activated" intermediates requiring ATP for their formation. There are two kinds of intermediates, namely (a) "activated foreign compound" as in hippuric acid synthesis and (b) "activated conjugating agent" as in glucuronide synthesis. The tissue distribution of these two kinds of intermediates is different, (a) being confined to the liver and/or kidney and (b) being more widely distributed.

280 Oxidation and Reduction by Microsomal Enzymes. J. R. Gillette (U.S.A.).

Most reactions by which lipid soluble drugs are oxidized in body are catalysed by enzyme systems localized in liver microsomes. These reactions comprise deamination of alkylamines, N-dealkylation of alkylamines, O-dealkylation of ethers, hydroxylation and perhaps epoxidation of aromatic compounds, hydroxylation of aliphatic compounds, and sulphoxidation of thio-ethers. The common requirements of the microsomal systems for TPNH and oxygen suggest that the enzymes utilize atmospheric O_2. The mechanism might be as follows: TPNH reduces a component of the enzyme system; the reduced component reacts with oxygen to form an "active oxygen donor" which oxidizes the drug substrate. In view of this proposed mechanism, it is important that the microsomal "drug enzyme systems" can be considered "hydroxylases". Attempts have been made to determine the nature of "active oxygen donor". Most of the reactions catalysed by the microsomes can occur with model systems. For example, a modified Fenton's reagent hydroxylates aromatic compounds and O-dealkylates ethers; horseradish peroxidase with hydrogen peroxide N-dealkylates alkylamines and sulphoxizes chlorpromazine. The presence of hydrogen peroxide in these systems suggested that "active oxygen donor" might be a peroxide. Although peroxide is formed during the oxidation of TPNH by microsomes, a peroxide generating system cannot replace the requirement for TPNH. Thus, the nature of "the donor" remains unknown.

Azo- and nitro-compounds are reduced to primary amines by enzyme systems in microsomes. Azo-reductase specifically requires TPNH and is only slightly affected by oxygen; but nitro-reductase can use either TPNH or DPNH and is blocked by oxygen. These enzyme systems are unusual in that their velocities approach infinity as the concentrations of both TPNH and substrate are increased. They are also unusual in that their activities are greatly enhanced by high concentrations of riboflavin, FMN or FAD. Presumably, the enhanced activity is due to a non-enzymatic reaction between the substrate and the flavin reduced by a microsomal system.

Animals use a number of these reactions to metabolize a particular drug. For example, chlorpromazine sulphoxidation, N-dealkylation and hydroxylation. Which of these reactions predominates depends on many factors including genetic background, sex and age of the animal.

281 Discussion of Previous Paper. K. Krisch (Germany).

282 Glucuronide Conjugation. G. J. Dutton (United Kingdom).

Apart from its occurrence in mucopolysaccharides, glucuronic acid forms simple glycosides with a wide variety of endogenous and foreign compounds, and the metabolic processes forming or breaking down these usually more readily eliminable glucosiduronic acids (or glucuronides) are of considerable pharmacological significance. Four main types of

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glucuronide are distinguishable, depending on the conjugated group of the aglycone, and one major biosynthetic pathway for three of them is now known.

The immediate donor of glucuronic acid to the aglycone is uridine diphosphate glucuronic acid (UDPglucuronic acid), arising from oxidation of UDPglucose, which in its turn has needed both uridine triphosphate and glucose-1-phosphate: (1) Uridine triphosphate + glucose-1-phosphate→ UDPglucose + pyrophosphate; (2) UDPglucose + DPN → UDPglucuronic acid + reduced DPN; (3) Aglycone + UDPglucuronic acid → Aglycone glucuronide + UDP.

Thus, energy and a source of glucose, for (1), and oxygen, for (2), are here required in the ultimate synthesis of the glucuronic acid moiety. By this means "ether", "ester" and *N*-glucuronides of both foreign and endogenous compounds can be formed. Whether the microsomal UDPtransglucuronylase ("glucuronyl transferase") catalysing reaction (3) is a single enzyme for all substrates, of whatever origin and structure, is not yet known; the evidence will be discussed.

Glucuronide synthesis occurs in livers of various species, and in several other tissues such as kidney, gastro-intestinal tract and skin. The significance of reactions (2) and (3) in these instances and differences due to developmental, sexual and genetic factors, together with attempts to stimulate conjugation, will be described. Evidence of other pathways will be reviewed, and the role of β-glucuronidase in glucuronide detoxication evaluated.

283 Sulphate Conjugation. J. D. Gregory (U.S.A.).

A number of sulphate esters are found as tissue components, and many others are normally excreted in the urine of most animal species. The conjugation of inorganic sulphate to form esters requires a preliminary conversion of the sulphate to a highly reactive form. The activation of each molecule of sulphate, at the expense of 2 molecules of ATP, results in the formation of 3′-phosphoadenosine 5′-phosphosulphate (PAPS). In all systems so far studied, PAPS is the point of departure for the synthesis of every kind of naturally occurring sulphate ester and also of the unnatural ones that result from the detoxication of substances administered to the organism.

The largest group of substances so far investigated is the phenols; phenyl sulphate, *p*-cresyl sulphate, indican, and tyrosine-*O*-sulphate, for example, are detectable in normal human urine. It has more recently been shown, in addition, that aromatic amines and aliphatic alcohols are also conjugated in mammalian tissues. Considerable progress has been made in isolating and characterizing the particular enzymes that transfer sulphate from PAPS to the various acceptors (sulphokinases).

The sulphate activating enzymes of yeast are conveniently used for investigation, but essentially the same reactions occur in liver, kidney, colonic mucosa, corneal epithelium, hen oviducts, and elsewhere. With some variations, the same tissues are sources of sulphokinases, although knowledge of the distribution and function of these enzymes is far from complete.

284 Discussion of Previous Paper. H. Boström (Sweden).

285 Mercapturic Acid Conjugation. E. Boyland (United Kingdom).

Many compounds are excreted as mercapturic acids which are derivatives of acetylcysteine. The metabolites of purely aromatic compounds such as naphthalene are *S*(α-β-dihydro-β-hydroxy-α-phenyl) acetylcysteine compounds which decompose in cold acid solution to give *S*-phenyl acetylcysteine derivatives. The metabolism of these aromatic compounds probably involves oxidation to intermediates with the properties of epoxides, which may react with water to give α,β-dihydro-α,β-dihydroxy derivatives or "diols", or with cysteine derivatives to give metabolites of the mercapturic acid type. Compounds which contain chemically reactive groups such as epoxide groups or chloro groups react directly with the cysteine derivative.

The formation of mercapturic acid involves the reaction of a chemically reactive compound with glutathione in the liver. The glutathione derivatives are excreted in bile but not in urine.[1] The glutathione derivatives appear to be converted to cysteine derivatives in the kidney and the final reaction which occurs in the liver is acetylation to yield the mercapturic acid which is excreted in urine. An enzyme, glutathione kinase, in the supernatant fraction of rat liver homogenate catalyses the reaction of organic compounds specifically with glutathione.[2]

Aniline and 2-naphthylamine are also excreted as mercapturic acids. 2-Naphthylamine is apparently converted to 2-amino-1-naphthyl mercapturic acid and *S*(2-amino-5:6-dihydro-6-hydroxy-5-naphthyl) *N*-acetyl cysteine among other products.[3] The first compound may arise by oxidation of 2-naphthylamine to 2-naphthyl hydroxylamine (RNHOH) which can react with glutathione (GSH) to yield a product $R(NU_2)(SG)$ which can be converted into 2-amino-1-naphthyl mercapturic acid which is excreted. The formation of the other mercapturic acid from 2-naphthylamine probably arises through the formation of the hypothetical 2-amino-5,6-dihydro-5,6-epoxy naphthalene.

Mercapturic acid conjugation is a general reaction by which foreign compounds which are either chemically reactive or insoluble are converted into inert water-soluble ionized compounds which can be excreted by the kidney. There are probably some types of foreign compounds which are conjugated as mercapturic acids still to be discovered.

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1. Boyland, Ramsay and Sims (1961).
2. Booth, Boyland and Sims (1961).
3. Manson (1961). Personal communication.

286 Discussion of Previous Papers. D. V. Parke (United Kingdom).

287 Mechanisms of Enzymatic Acylation and Peptide Bond Formation. A. Meister (U.S.A.).

This paper will consider some recent studies on enzymatic acylation and peptide bond formation with particular reference to the mechanisms of activation of carboxyl groups. One type of activation is accompanied by cleavage of ATP to AMP and pyrophosphate, and involves intermediate formation of enzyme-bound acyl adenylate. Several examples will be discussed, including enzymatic reactions leading to the synthesis of carnosine, anserine, related γ-aminobutyryl and β-alanyl peptides, phenylacetylglutamine, and hippuric acid. Another category includes activation reactions associated with cleavage of ATP to ADP, and inorganic phosphate, e.g. glutamine synthesis. Recent experiments on the mechanism of glutamine synthesis provide evidence for a new type of activation involving an enzyme-bound form of glutamic acid. A general scheme to explain the several catalytic activities of this enzyme has been formulated. The possibility that analogous mechanisms may account for certain other types of activation will be discussed.

288 Effects of Drugs on Synthesis and Mobilization of Lipids. E. C. Horning (U.S.A.).

289 Various Mechanisms Affecting Bile Acid Metabolism. S. Bergstrom (Sweden).

A review will be given of the degradation of cholesterol to bile acids and the technique by which this process can be followed quantitatively. The influence of various factors on this process will be illustrated and discussed.

290 Effects of Major Nutrients on Human Lipid Metabolism. E. H. Ahrens, jr. (U.S.A.).

Recent experiments will be presented which pertain to two lines of study in man: (1) the use of synthetic triglycerides as dietary fats to elucidate whether and to what extent the various types of fatty acids (saturated, monoenoic and dienoic) affect serum lipid levels; and (2) the induction of visible lipaemia in patients fed high-carbohydrate, low-fat diets, and evidence obtained relative to the source of endogenous chylomicrons. The relevance of such studies to current concepts of atherogenesis will be noted.

291 Drugs and Hormones affecting Serum Lipid Levels. D. Steinberg (U.S.A.).

Because of the probable relationship between elevated serum lipid levels and the development of atherosclerosis there is growing interest in the possible contributions pharmacology can make in this area. An attempt is made to outline the feasible chematherapeutic approaches to control of serum lipid levels and to discuss the rationale of each in the light of current understanding of lipid biochemistry and physiology. The large gaps in our knowledge, especially with respect to the basic mechanisms of lipoprotein production and lipoprotein degradation, are pointed out. The pros and cons of various drug screening methods are discussed.

The approach based on inhibition of cholesterol biosynthesis is treated in more detail, using the "case history" of triparanol (MER-29) for illustration. This compound has a well-defined mechanism of action and has been proved to be clinically effective. It acts by inhibiting the reductive conversion of 24-dehydrocholesterol (desmosterol) to cholesterol. As a result of this inhibition there is accumulation of desmosterol in serum and tissue but total sterol levels are nonetheless reduced. There is no doubt that an effective inhibition of cholesterol synthesis can reduce serum sterol levels. Experience with other inhibitors is briefly reviewed and some of the problems that have been encountered with them are discussed.

Evidence has accumulated recently to show that emotional stress can produce hypercholesterolaemia. A hypothesis is presented, based on "model" studies in experimental animals, to the effect that this hypercholesterolaemia may be secondary to combined overactivity of the adrenal cortex and the adrenal medulla and mediated through the mobilization of free fatty acids from the periphery.

292 Cholesterol Metabolism in Man. L. I. Gidez, H. A. Eder (U.S.A.).

[2-^{14}C] mevalonic acid was administered to patients. Free and esterified cholesterol were isolated from serum and the various lipoprotein fractions, and the specific radioactivity was determined. In contrast to free cholesterol, the specific activity of the cholesterol esters of the various lipoproteins differed: the highest activity was in the D < 1·019 and D 1·063–1·21 fractions. The radioactivity in the D 1·019–1·063 fraction was consistently lower. One patient demonstrated a double specific activity peak in the low density lipoproteins. These curves may be the resultant of two curves, one representing cholesterol ester synthesis in liver, the other representing cholesterolester synthesis in an extrahepatic site. The differences in the specific activity of the esterified cholesterol at all times up to two days after isotope administration are consistent with the *in vitro* finding that cholesterol esters do not exchange between the different lipoprotein fractions. It was assumed that all the radioactive carbon which was not excreted in urine or as CO_2 was incorporated into cholesterol. From this estimate of radioactivity incorporated into chol-

esterol and the zero-time specific activity obtained by extrapolation of the linear portion of the specific activity-time curve, the total body pool of cholesterol was estimated. From the slope of the linear section of the curve, the fractional turnover rate was calculated, and in three patients this proved to be 0·7, 1·8 and 1·2 per cent per day. From this it was calculated that these patients synthesized a maximum of 1·5, 3·9 and 3·8 g of cholesterol per day respectively.

293 Effect of Dietary Fat on Serum Cholesterol Levels and Bile Acid Turnover in the Rabbit. K. Hellström, J. Sjövall and G. Wigand (Sweden).

White rabbits of the same strain weighing between 2 and 3 kg were provided with a stomach fistula. This consisted of a PVC plastic tube which permitted bile sampling when a rubber tubing was introduced into the duodenum. Except when samples were taken the fistula was stoppered and the animals moved freely in their cages. Deoxycholic acid-24-^{14}C was given intraperitoneally and bile samples were taken after the injection of cholecystokinin at intervals of 2–5 days. The specific activity of the biliary glycodeoxycholic acid was determined by quantitative paper chromatography and liquid scintillation counting of the spots on the chromatograms. Thus, the half-life and pool of bile acids could be determined. The rabbits were fed an isocaloric pellet food and had free access to water. On "standard rabbit food" (10 cal % fat) the bile acid pool was 640 mg (281–1008), the half-life 6·6 days (3·4–9·6) and the daily bile acid production 72 mg (57–88) in 11 rabbits. When the diet was changed to pellets containing 26 cal % of hydrogenated coconut fat the decrease of the specific activity of glycodeoxycholic acid immediately changed to a slower rate. One rabbit after being fed the coconut diet for two months had a bile acid pool of 2·1 gm and the half-life of glycodeoxycholic acid was 31 days. Similar experiments with a corn oil diet are in progress and the results will be reported. The relationship between the changes in serum cholesterol and the bile acid turnover will be discussed.

294 Studies on the Mechanism of Action of Cholesterol Synthesis Inhibitors. W. L. Holmes (U.S.A.).

Investigations in a number of laboratories during the past three years have revealed several classes of compounds which interfere with the conversion of mevalonic acid to cholesterol *in vitro* and/or *in vivo*. This report is concerned with comparative studies of the mechanism of action of a number of these inhibitors. Included in this group are N-(1-methyl-2:3-di-*p*-chlorophenylpropyl)-maleamic acid (Benzmalacene), (*p*-(β-diethylaminoethoxy)-phenyl)-1-(p-tolyl)-2-(*p*-chlorophenyl) ethanol (Triparanol), β-diethylamino-ethyldiphenylpropyl acetate hydrochloride (SK&F 525-A), 2:2-diphenyl-1-(2-dimethylaminoethoxy)-pentane hydrochloride (SK&F 3301-A), and (tris-(2-di-methylaminoethyl)-phosphate) hydrochloride (SK&F 7723-A[7]).

Evidence has been obtained which suggests that the hypocholesterolemic agent, SK&F 7732 and its diethyl analogue, inhibit cholesterol synthesis *in vitro* at a site lying between lanosterol and zymosterol. SK&F Nos. 525 and 3301, benzmalacene, and to a lesser extent triparanol have been shown to inhibit cholesterol synthesis *in vitro* at the polyprenol purophosphate level. The first two compounds also produced a major block at this level *in vivo*. SK&F Nos. 525 and 3301, and triparanol have been found to interfere to some extent with cholesterol biosynthesis *in vivo* at two other sites, i.e. the cyclization of squalene and between lanosterol and zymosterol.

Recently developed methods of gas chromatography have been used to investigate the sterol content of the non-saponifiable fraction of several different tissues from pretreated animals. These studies are of particular interest since they readily demonstrate the applicability of gas chromatography to mechanism studies in this field. Despite the structural similarity of some of the compounds under study only triparanol was found to induce an accumulation of sterols other than cholesterol in rat tissues.

295 The Metabolism of Desmosterol in Human Subjects During Triparanol Therapy. DeW. S. Goodman, J. Avigan and H. N. Wilson (U.S.A.).

The conversion of 24-dehydrocholesterol (desmosterol) to bile acids and to steroid hormones during triparanol administration has been studied in two male subjects. In the first study, DL-2-^{14}C-mevalonic acid was administered intravenously and the ^{14}C specific activities of plasma desmosterol and cholesterol determined after 3 hr and 3 days. At 3 days, bile was collected by duodenal intubation and the cholic acid purified by extraction and chromatography of the bile acids. A 24-hr urine sample was also obtained and the urinary derivatives of cortisol extracted and purified by chromatography in two systems. Considerable ^{14}C activity was observed in both the cholic acid and the cortisol derivatives, with specific activities indicating that desmosterol had been a satisfactory precursor for these compounds.

A second and more elaborate study was then conducted in which the subject received an intravenous injection containing both 7-alph-^{3}H-cholesterol, previously incorporated into his own serum lipoproteins, and 2-^{14}C-mevalonic acid. This resulted in a labelling of the plasma-liver cholesterol pool with ^{3}H, and of the corresponding desmosterol pool with ^{14}C. Serial blood, bile, and urine samples were obtained at one- or two-day intervals for one week. Specific activity curves for

^{3}H and ^{14}C were then determined for both free and esterified desmosterol and cholesterol. The curves for the two sterols were very similar, with desmosterol disappearance being slightly more rapid than that of cholesterol. This difference could be explained by the slow conversion of desmosterol into cholesterol, a reaction which could be quantitated from the rate of appearance of ^{14}C in cholesterol, combined with the disappearance rate of ^{3}H-cholesterol. The ^{14}C specific activity curve of the purified bile acids was determined and compared with the plasma sterol curves; this showed desmosterol to be an excellent precursor for bile acids, quantitatively quite comparable to cholesterol. A similar conclusion was reached about the ability of desmosterol to act as a precursor of cortisol and of androgenic steroids. This was determined by isolation and purification of the cortisol derivatives and of the androgenic steroid derivatives for the several urine specimens. Specific activity curves for both ^{3}H and ^{14}C for these two kinds of steroids were constructed. Both isotopes were present in significant amounts in all samples, with only minor changes in the isotope ratio being observed during the course of the study. These results demonstrate that desmosterol is quantitatively very similar to cholesterol in serving as a precursor for several important metabolic products of cholesterol, namely bile acids, cortisol, and androgenic steroids.

296 Oxidation of Cholesterol-26-^{14}C by Rat Liver Mitochondria: Influence of Sex and Sex Hormones. D. Kritchevsky, M. W. Whitehouse and E. Staple (U.S.A.).

A summary of a number of experiments has shown that female rat liver mitochondria consistently oxidize more cholesterol-26-^{14}C than do male rat liver mitochondria. In one series of experiments 4 groups of male rats were treated as follows: male control (M); castrated (MC); oestrogenized (15γ oestradiol benzoate/150 g/day) (ME); and castrated oestrogenized (MEC). In a series of experiments the castrated or oestrogenized males oxidized more cholesterol-26-^{14}C to $^{14}CO_2$ than did the male controls. In another series, 4 groups of female rats were used: female control (F); gonadectomized (FG); androgenized (1 mg testosterone propionate/150 g/day) (FA); and gonadectomized androgenized (FGA). The gonadectomized rats oxidized more cholesterol-26-^{14}C to $^{14}CO_2$ than did the female controls. This enhancement of oxidation was not observed with the other two groups (FA, FGA).

In the male series, serum and liver cholesterol levels were significantly elevated in the 3 "feminized" groups. In the female series, there was a rise in serum cholesterol levels in the gonadectomized group, while serum cholesterol levels decreased in the other two groups. Liver cholesterol levels were substantially unaltered in all 4 female groups.

(Supported by NIH Grant H-3299.)

297 The Actions of Testosterone in Cholesterol-Fed Cockerels. W. L. West, H. Y. C. Wong and T. F. Johnson (U.S.A.).

The effects of testosterone propionate, androsterone and 17-ethyl-19-nortestosterone were compared in cockerels on a high cholesterol diet. Both testosterone and androsterone produced significant inhibition of experimental atherosclerosis while 17-ethyl-19-nortestosterone did not. The effect on blood cholesterol levels and phospholipids were not significantly different in the groups studied. Testosterone and androsterone significantly increased the rate of absorption of orally administered ^{14}C-cholesterol from the gastro-intestinal tract when compared with experimental controls. When ^{14}C-cholesterol was given intravenously, there was a significantly greater loss of labelled metabolites in the testosterone and the androsterone-treated birds when compared with experimental control. Further studies are in progress in an effort to relate the results obtained with testosterone in the experimental atherosclerosis of cockerels to the anabolic or some property of testosterone or its metabolites.

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298 Mechanism of Emesis and Anti-Emesis. (Film.) S. C. Wang (U.S.A.).

299 The Isolation of Nerve-endings from the Central Nervous System. (Film.) V. P. Whittaker (United Kingdom).

300 The Buffering of Carbondioxide during Hypercapuic Acidosis. (Film.) G. G. Nahas, S. J. Dos, J. Strauss and M. H. Holmdahl (U.S.A.).

301 Brain and Social Behaviour in the Monkey (Film). J. M. R. Delgado (U.S.A.).

The purpose of this film is to demonstrate techniques to quantify social behaviour of monkeys and to study modifications induced by drugs and by brain stimulation. The first part explains the methods used: (1) to record social behaviour by time-lapse photography; (2) to analyse and quantify the records by means of a time-study projector, electric typewriter, and electronic computer; and (3) to programme stimulation of the animals with tiny transistor-stimulators carried on a collar and connected to the brain through subcutaneous leads. The second part of the film shows quantification of 24 categories of daily behaviour. Administration of chlorpromazine, reserpine, iproniazid, pentobarbital and oestradial modify the quality and quantity of behavioural categories. Stimulation of central grey increases the aggressiveness of the stimulated monkey and produces social spread of attacking behaviour. Stimulation of

motor areas does not disturb the animals. Stimulation of the nucleus anterior ventrails of the thalamus evokes running away and hiding. Histological study shows that prolonged stimulation of the brain does not alter the neurons.

302 Methods of Discovering Thrombotic and Antithrombotic Substances. Discovery of a New Type of Antithrombotic Effect of Monoamine Oxidase (MAO) Inhibitors. (Film.) T. SHIMAMOTO (Japan).

303 The Relationship between the Chemical Structure and Pharmacological Activity of some Esters of 3-Oxyquinuclidine. M. D. MASHKOVSKY (U.S.S.R.).

The pharmacological properties of esters of 3-oxyquinuclidine with fatty, aromatic, fatty-aromatic and heterocyclic acids, of 3-oxyquinuclidinecarbamic esters and their quarternary derivatives were studied. These preparations were synthetized by Rubtsov *et al.*

3-Acetoxyquinuclidine was found to be a highly active cholinomimetic agent. The cholinomimetic properties of this compound depend on the presence of the quinuclidine heterocycle in its molecule; its N-methyl-piperidine analogue is much less active. The pharmacological action of 3-acetoxyquinuclidine also depends on the presence of the tertiary nitrogen atom in its molecule; indeed, quaternization considerably reduces its activity. The lengthening of the radical, which esterifies 3-oxyquinuclidine causes the disappearance of cholinomimetic properties and the appearance of cholinolytic activity. 3-Benzoyloxyquinuclidine has a weak cholinolytic activity, but possesses a hypotensive and sedative action. This action appreciably diminishes when substituents are introduced into the benzene ring.

The carbamates of 3-oxyquinuclidine possess a very slight activity. Methiodide of 3-dimethylcarbamyl-oxyquinuclidine has a weak nicotine-like action. The esters of 3-oxy:3-alkyl(aryl)quinuclidine have no apparent activity.

304 Optical Isomerism and Cholinomimetic Activity. B. V. RAMA SASTRY and J. V. AUDITORE (U.S.A.).

Published literature on the relationships between the chemical constitution and the biological responses of cholinomimetics and atropine-like compounds suggest that an acetylcholine-like neurohumour with an asymmetrical centre in the alpha position to the carbonyl group may occur in nature.[1] To test whether L(+)-lactoylcholine (LCh) will satisfy the requirements of such a neurohumour, the pharmacodynamic evaluation of D(−)- and L(+)-LCh was undertaken. The isomeric ratios (I.R.) between the enantiomers of LCh (D(−)/L(+): dog B.P. 11·4, dog B.P. after atropine 5·0, guinea-pig ileum 5·0, rabbit intestine 5·0, frog rectus abdominis muscle 2·0) indicate that asymmetry in the acyl component of cholinesters is a significant factor in the muscarinic and nicotinic effects of cholinomimetics. Our results indicate that erythrocyte and plasma cholinesterases (ChE) from human and dog hydrolyse L(+)-LCh at a rate faster than that of ACh and show preferential affinities for L(+)-LCh (I.R. L(+)/D(−): human RBC 5·0, human plasma 1·5, dog RBC 8·6, dog plasma 1·7). The close fit of L(+)-LCh to the asymmetric area at the esteratic site and the high electrophilic nature of its carbonyl carbon may contribute to its rapid hydrolysis by ChE. The stereospecificity of specific ChE seems to have a greater significance than that of plasma ChE for this pair of enantiomers. Estimation of the muscarinic and nicotinic activities (dog B.P. before and after atropine) in the light of their rates of hydrolysis by dog RBC and plasma ChE seem to indicate that: (1) L(+)-LCh is more active than D(−)-LCh in its muscarinic and nicotinic activities, and (2) L(+)-LCh is about four times more active than ACh in its nicotinic action and is about 75 times less active than ACh in its muscarinic action. Our results show that muscarinic receptors, nicotinic receptors and cholinesterases distinguish between these enantiomorphic pairs of cholinesters containing an asymmetric carbon in the alpha position to the carbonyl group.

1. SASTRY, PFEIFFER and LASSLO (1960), *J. Pharmacol.*, **130,** 346.

305 The Isolation of Radioactive Phosphorylserine ($Ser^{32}P$) from Different Rat Liver Cell Fractions Incubated with ^{32}P-Labelled Di-isopropyl Fluorophosphonate ($DF^{32}P$). G. ÅGREN and L. ENGSTRÖM (Sweden).

Di*iso*propyl fluorophosphonate (DFP) is known to inactivate several enzymes by reacting with their active sites.[1] After acid hydrolysis of the inactivated enzymes phosphorylserine can be isolated as a characteristic degradation product. Assuming that other enzymes may also behave in the same way, we were interested in determining the amount and distribution of DFP-reacting proteins in the rat liver cell fractions, obtained according to Hogeboom and Schneider.[2]

The cell fractions were incubated for 30 min with different concentrations of $DF^{32}P$ at 0° and pH 6·8. The medium contained 0·25 M sucrose, 0·01 M sodium phosphate, pH 6·8, and 2·5 per cent propylene glycol with $DF^{32}P$ added to a final concentration of 10^{-2}–10^{-6} M. The incubation mixtures were precipitated by trichloroacetic acid. The precipitates were dried with ethanol and ether. After acid hydrolysis $Ser^{32}P$ was isolated by chromatography on Dowex 50 as previously described.[3]

The amount of Ser^{32}P from the fractions was estimated from its total radioactivity divided by the specific activity of the DF^{32}P used. The result is given in Table I.

Table I

Amounts of Ser^{32}P isolated from different rat liver fractions after incubation with DF^{32}P, calculated as μmoles per g dry weight.

	Molar concentration of DF^{32}P				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Nuclear fraction	0·08	0·02	0·05	0·04	0·04
Mitochondrial fraction	0·04	0·02	0·06	0·02	0·01
Microsomic fraction	0·21	0·16	0·15	0·13	0·08
Supernatant fraction	0·03	0·07	0·01	0·01	0·02

According to our experience only about 20–25 per cent of acid-stable phosphoprotein phosphorus can be isolated as phosphorylserine. With this fact in mind, the amounts of Ser^{32}P isolated seem to be fairly large, especially from the microsome fractions, where the Ser^{32}P isolated then corresponds to about 0·3–1·0 μmole of protein-bound phosphorus per g dry weight.

With regard to the low DF^{32}P concentrations used in some of the experiments, it does not seem improbable that most of the Ser^{32}P derives from enzymatically active proteins.

1. Holmstedt, B. (1959), *Pharmacol. Rev.*, **11**, 586.
2. Hogeboom, G. H. and Schneider, W. C. (1952), *J. Biol. Chem.*, **197**, 611.
3. Ågren, G., de Verdier, C.-H. and Glomset, J. (1954), *Acta Chem. Scand.*, **8**, 503.

306 Analysis of Purified Plasma Cholinesterase by Electrophoresis and Kinetic Studies. E. Heilbronn (Sweden).

Kinetic results obtained with human plasma fraction IV-6-3 can be explained by assuming the occurrence of two cholinesterases.[1] Further studies on purified horse plasma show that after paper electrophoresis, two zones are obtained which stain with indoxylacetate. No colour develops after treatment with eserine. The p*S*-activity curves again follow the Michaelis–Menten theory only if two simultaneously acting cholinesterases are assumed. Studies with a reversible inhibitor, 1,2,3,4-tetrahydro-5-aminoacridine, also point towards the presence of two enzymes in the preparation. Efforts are made to separate the enzymes by column electrophoresis. The separated fractions are analysed for differences in their behaviour towards substrates and inhibitors.

1. Heilbronn, E. (1958), *Acta Chem. Scand.*, **12**, 1878.

307 The Behaviour of ACHE in the Optic Lobes after the Removal of the Eye in the Chick. G. Filogamo, G. Gabella and E. Strumia (Italy).

In previous experiments, the extirpation of the right eye in the chick, 24–48 hr after hatching, resulted in transient decrease in the intensity of the Koelle reaction for ACHE in the controlateral optic lobe (two days after removal) followed by a speedy recovery; only after 50–60 days was the reaction gradually seen to lose its intensity at the same rate as the hypotrophy of the neurons in the same optic lobe.

In the present experiments, 2–6 mg of DFP, either immediately or at different time intervals after eye extirpation, were injected subcutaneously. Both histochemical and biochemical investigations have shown a marked decrease in ACHE activity after the first 24 hr in both lobes. In the days following this treatment, the enzyme's recovery became constantly slower and less complete in the lobe lacking retinal fibres than in the normally connected one. A general interpretation of the experimental results will be presented.

308 The Influence of Chemical Structure on the Interaction of Gamma-Aminobutyrylcholine Derivatives and Human Cholinesterases. F. F. Foldes and V. M. Foldes (U.S.A.).

The interaction of 16 γ-aminobutyrylcholine derivatives with human cholinesterases was investigated. The general formula of the compounds was $H_2N—(CH_2)n—OC—O—CH_2·N(CH_3)_3$. None of the compounds was hydrolyzed by human red-cell cholinesterase (RChE). The hydrolysis rate of the unsubstituted compounds by concentrated, purified human plasma cholinesterase (Cholase; PChE) was 5·4, 6·9, 66·9, 103·7 and 91·2 μM/ml plasma/30 min when n equalled 3, 4, 5, 6 and 7 respectively. Gradual substitution of the H atoms of the H_2N radical by CH_3 groups decreased the hydrolysis rate of the compounds in proportion to the number of the CH_3 substitutions. The RChE inhibitory effect of 10^{-3} M concentrations of the unsubstituted compounds increased with increasing values of n, and ranged from 5 per cent (n = 3) to 57 per cent (n = 7). Inhibition was the greatest with the dimethyl substituted compounds ranging from 12 per cent (n = 3) to 96 per cent (n = 7). The PChE inhibitory effect of the unsubstituted compounds was insignificant. However, when n = 4 or 5, the dimethyl substituted compounds in 10^{-3} M

concentration caused 72 per cent inhibition. Several of the compounds produced a depolarizing, neuromuscular block in both cat[1] and man. In man, the intensity and duration of the block was proportional to the enzymatic hydrolysis rate of the compounds.

(1. Personal communication from B. B. Brown and L. E. Bailey, Riker Laboratories, Inc., Northridge, California.)

309 Some Difficulties of the Rigid Application of Structure–Activity Relationships. F. H. Shaw (Australia).

The substance tetrahydro-5-aminoacridine is both a powerful anti-cholinesterase and mild anti-choline-acetylase. As would be expected, it resembles eserine or rather neostigmine in its pharmacological effects. It reverses the NMJ block produced in man and animals by tubocurarine and gallamine and potentiates the block produced by succinylcholine; it is used for such purposes clinically. Whilst in man it does not have a cholinergic effect on the intestine, salivary or bronchial secretion and cardio-vascular system, it does in most laboratory animals profoundly affect these systems in the expected manner. On the other hand, it scarcely antagonizes the effect of tubocurarine, etc. on the phrenic nerve-diaphragm preparation (rat). Thus, the first problem is to account for a marked species variation, which is nowhere near as marked with other anti-esterases. But tetrahydroaminacridine possesses additional properties not found with other anti-cholinesterases (which might be accounted for by its anti-acetylase activity) viz. respiratory stimulation and restoration of consciousness in narcotized and uraemic animals and man.

Amiphenazole (2-4 diamino-5-phenylthiazole), which has no anti-enzyme activity, possesses exactly the same stimulating action on the higher nervous centres but no eserine-like activity at the NMJ. Thus we have to explain similarity of pharmacological effects (on the CNS) without being able to have recourse to either similarity of structure or (so far as is known) identity of biochemical activity. Other instances of the similarities and differences of activity of these two substances (and related ones) will be given.

310 The Role of the Onium Group in the Molecule of an Anticholinesterase in its Penetration through the Blood–Brain Barrier (BBB). M. J. Michelson, E. V. Zeimal, L. G. Magazanik, M. I. Kabachnik and N. N. Godovikov (U.S.S.R.).

Anticholinesterases with general formula: $H_5G_2O(CH_2)P(O)SCH\text{-}CH_2R$; R = -S-$C_2H_5$(Gd-7); R = -S$^+$$(CH_3)C_2H_5OSO_3OCH_3$ (Gd-42); R = -S-$C_{10}H_{21}$(Gd-107); R = -S$^+$$(CH_3)C_{10}H_{21}$. OSO_2OCH_3(Gd-108) were synthetized. The concentrations of cholinesterase inhibitors in different tissues *in vivo* after i.v. injection were determined by means of calibrating curves where the percentage of cholinesterase inhibition in corresponding tissues *in vitro* was plotted against the drugs concentration. Gd-7 easily penetrates into the brain. The concentration of Gd-42 in the brain is 10 or more times less than in the muscles of the cat, chicken and frog.

When 1 DL of Gd-42 (0·005 mg/kg) was injected i.v. in the cat, no drug could be detected in the brain. Only when 10 LD of Gd-42 (0·05 mg/kg) were injected i.v. significant concentrations of the drug were found in the brain. In this case no statistically significant difference in the concentrations of the drug in the cortex, cerebellum, middle brain, caudate nucleus and medulla were revealed. In Cyclostomata (*Petromyson fluviatilis*) the BBB for onium compounds is relatively weak, but the difference in their concentration in the spinal cord and the muscles still exists.

In the embryogenesis of chicken on the tenth day of incubation, the BBB for onium salts does not exist; the concentrations of Gd-42 are equal in the brain and the muscles.

The substitution of the ethyl group connected with the sulphonium sulphur atom by a decyl group (Gd-108) does not facilitate the penetration through the BBB, but even makes it more difficult. Its non-ionized analogue Gd-103 penetrates through the BBB without any difficulties.

311 The Role of Cholinergic Systems of the Brain Stem Reticular Formation in the Mechanism of Central Effects of Anticholinesterase and Cholinolytic Drugs. R. J. Ilyutchenok (U.S.S.R.).

The problem of availability and role of cholinergic systems in the reticular formation of the brain stem has not been closely studied. Scientific researches carried out by us by applying various pharmacological drugs and recording the brain electrical activity in experimental animals show that cholinergic systems are of great importance in the activity of the reticular formation and that the mechanism of central action of a number of anticholinesterase and cholinolytic drugs is closely connected with the influence upon these systems. The administration of anticholinesterase drugs induced changes in the electrical activity of the cerebral cortex, non-specific nuclei of the thalamus and reticular mesencephalic formation in experiments on rabbits and cats. Observed EEG activation was easily blocked by central cholinolytic drugs (amisil-benactyzine) but not by peripheral cholinolytics (metacine) and chlorpromazine. In cats with "cerveau isole" preparation and those with sections at the level of the superior c. quadrigemina and pons anticholinesterase drugs have the same effect as upon the intact animal. All these effects could be abolished by amisil but not by chlorpromazine. Under this condition after amisil administration, EEG activation is not observed even when overdosing with

galantamin and eserine; but following the administration of phenamine (amphetamine) low amplitude fast activity was induced after sectioning along the line connecting the superior c. quadrigemina and c. mamillaria and more rostrol sectioning the administration of eserine, galantamin and phenamine did not induce the EEG activation. Thus, we observe the EEG activation after galantamin and eserine injection when the cerebral cortex is connected with the midbrain. These experiments in sectioning the brain stem at different levels suggest that the activating effect of anticholinesterase drugs depends on their influence upon the midbrain reticular system and this fact shows the availability of cholinergic structures in it and their role in the function of the reticular formation. The blocking effect of amisil upon the EEG activation produced by anticholinesterase drugs, and the absence of such an effect with metacine and chlorpromazine show an interaction between the central cholinolytic and cholinoreactive systems of the reticular formation.

312 A Pharmacological Demonstration of Cholinergic Mechanisms in the Central Nervous System. L. S. Harris (U.S.A.).

Previous studies, utilizing the photocell activity cage, have shown that various anti-cholinergic agents, scopolamine, atropine, trihexyphenidyl, etc. produce an increase in the spontaneous activity of mice.[1] The quaternary salts, which do not readily cross the blood-brain barrier, do not share this activity.

Tremorine, which probably acts by cholinergic mechanisms, produces a decrease in spontaneous activity at non-tremorigenic doses. In addition to antagonizing the tremors produced by Tremorine, the centrally acting anti-cholinergics specifically reverse the depression of spontaneous activity produced by this drug. The quaternary salts are unable to produce this reversal.

Other non-quaternary cholinergic agents such as pilocarpine, and 2-dimethylaminoethylacetate (the tertiary amine of acetylcholine), also cause a depression of spontaneous activity and, at some doses, tremors in mice. The tremors and depression of activity produced by these compounds can be reversed by the centrally active anti-cholinergics. In addition, small doses of the cholinesterase inhibitor eserine produce a depression of activity. This effect is obtainable in the presence of amounts of atropine methyl nitrate which completely protect against the peripheral actions of eserine. Again, the depressant effects of 2-dimethylaminoethylacetate are markedly potentiated by eserine.

Thus, cholinergic agents and the anti-cholinesterase eserine, which have access to the central nervous system, depress the spontaneous activity of mice. The anti-cholinergic agents stimulate the activity and readily reverse the depression produced by the cholinergic agents and Tremorine. These findings will be discussed in relation to cholinergic mediation in the central nervous system.

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1. Harris, L. S. (1961), *Fed. Proc.*, **20,** 395.

313 Amphetamine Induced Recovery of Conditioned Reflexes disturbed by Cholinolytics and Chlorpromazine. M. N. Linuchev, N. J. Lucomskaja, M. J. Michelson and E. L. Shchelkunov (U.S.S.R.).

Different cholinolytics induce disturbances in the nervous activity of men and animals which can be reduced or completely prevented by simultaneous injection of anticholinesterases: eserine, parooxon, armine, prostigmine (Michelson *et al.*, 1954, 1957, 1959). Such antagonism could probably occur at the level of cholinergic structures participating in the performance of nervous activity.

In healthy men the temporary disturbance in mental activity induced by parpanite could be reduced by the preliminary taking of amphetamine in therapeutic dose.

The disturbance in motor–food conditioned reflexes of rats in a labyrinth was induced by atropine, parpanite and other cholinolytics. The preliminary or simultaneous injection of an adequate dose of amphetamine (0·5–2·0 mg/kg) completely or partly prevented the disturbance caused by cholinolytics.

The disturbance in the motor–food conditioned reflexes of rabbits and cats caused by benactyzine could be reduced by injection of adequate doses of amphetamine.

Amphetamine restored to normal the motor-food and avoidance conditioned reflexes of rats disturbed by chlorpromazine. This antagonism occurred even in cases when a large range of doses of amphetamine and chlorpromazine, respectively, had been used.

The ability of ayphetamine to reduce the disturbances which cholinolytics caused in nervous activity probably could be explained as a manifestation of adaptative adrenergic influence on cholinergic brain synapses. Special features of the antagonism of chlorpromazine and amphetamine suppose another level, e.g. the adrenergic structures of the brain stem reticular formation.

314 Relationship between Central Anticholinergic Actions and Antiparkinson Efficacy of Phenothiazine Derivatives. R. P. White and E. J. Westerbeke (U.S.A.).

Ethopropazine (Parsidol) is employed clinically as an antiparkinson agent, whereas promethazine, thioridazine, promazine, prochlorperazine are not and, with the exception of several, have been reported to produce extrapyramidal overactivity toxic effects. Since Parkinsonism may result from an over-active cholinergic mechanism,[1] it seemed

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of interest to ascertain whether Parsidol would preeminently block the EEG activation produced by low doses (0·1 mg/kg) of physostigmine or di*iso*propylfluorophosphate (DFP) in unanaesthetized rabbits. In comparatively low dosage (1–2 mg/kg) intravenously, all of the phenothiazines induced a synchronous EEG pattern which was converted to an alert tracing by the cholinergic drugs. In higher doses (10 mg/kg), however, only Parsidol blocked completely the EEG effects of physostigmine or DFP indicating, together with marked mydriasis, significant anticholinergic actions.

Experiments with D-amphetamine (1–5 mg/kg) show that all of the phenothiazines tested reduce or antagonize the EEG and behavioural effects of this drug and that no marked distinction exists between the phenothiazines in this regard. Hence, the pharmacological data suggest that the antiparkinson action of Parsidol is exerted by means of a central anticholinergic effect. The report of White *et al.*[2] showing that hyoscine and atropine, in that order of potency, block the EEG action of physostigmine and that chlorpromazine failed to manifest such a blockade further supports this suggestion.

(Supported by U.S.P.H.S. Senior Research Grant SF-279.)

1. (1956), *Ann. New York Acad. Sci.*, **64,** 690.
2. White and Boyajy (1960), *Arch. int. Pharmacodyn.*, **127,** 260.

315 Effect on Plasma Catecholamine Concentration of Haemorrhage and pH Alteration Followed by Various Forms of Therapy. W. M. Manger, G. G. Nahas, D. Hassam and S. Dufton (U.S.A.).

Haemorrhage of third or more of total blood volume in dogs was accompanied by increase in concentration of plasma catecholamines which was correlated with quantity of blood loss and severity of hypotension. Usually increase in epinephrine was considerably greater than norepinephrine. Slow haemorrhage caused smaller increases in catecholamines than rapid removal of the same quantity of blood. Further increases in catecholamines accompanied prolonged haemorrhagic hypotension. It seemed the principal factor activating the sympathetico-adrenal system was hypotension. However, hypotension following intravenous administration of histamine or egg white to sensitized dogs almost invariably caused smaller increases in catecholamines than did the same degree of hypotension following haemorrhage.

Replacement of blood loss with herparinized blood (even in dogs whose blood pressure was maintained at 50 mm Hg for 2¼ hr) usually resulted in restoration of blood pressure and catecholamines towards normal. Following rapid haemorrhage of about half blood volume, rapid transfusion with blood collected in acid citrate dextrose (A.C.D.) solution resulted in severe acidosis and death in 90 per cent of dogs. Catecholamines were increased significantly during haemorrhage and transfusion—particularly in animals when acidosis was most severe. If A.C.D. blood was titrated with trihydroxymethyl aminomethane (THAM) to an average pH of 7·56, rapid transfusion resulted in survival of 80 per cent of animals and return of catecholamines to about control levels.

Transfusion acidosis can also be prevented by maintenance of relatively normal arterial pH with addition of Na_2CO_3. Simultaneous administration of $CaCl_2$ or addition of large concentrations of catecholamines to acid blood will also protect dogs against transfusion acidosis, mostly by maintaining adequate cardio-vascular function.

316 Importance of Bulbar Autonomic Mechanisms in the Pentylenetetazol-Induced Cardiac Arrhythmias and Hypertension in Dogs. S. C. Wang, R. P. Bircher and T. Kanai (U.S.A.).

It has been previously ascertained by stimulation and transection experiments that the sympathetic pressor centre is located in the periventricular area at the level of the medulla oblongata, and the vagal and sympathetic depressor centre is located caudally in the obex. Contrary to the recent claim of Peiss (1960) that reflex cardio-accelerator mechanism is located in the hypothalamus, our evidence indicates that cardio-acceleration can be reflexly elicited in animals without hypothalamus, and that the reflex mechanism for cardio-acceleration is also located in the medulla, in the same general region for the sympathetic pressor responses. These bulbar sympathetic and vagal mechanisms are important for the tachycardiac or bradycardiac arrhythmias and hypertension, induced by pentylenetetrazol given to succinylcholinized animals intravenously or into various cerebral ventricles. These cardiac responses can be readily prevented by protecting the heart from the effects of centrally mediated vagal and sympathetic outflows (vagotomy, l-hyos-cyamine or N-ethyl-nortropine-benzydryl-ether; and complete sympathectomy, cervical chordotomy or dihydrogenated ergot alkaloids). The hypertensive responses, on the other hand, are not completely prevented by the above procedures. This evidence suggests the possibility of some constrictor action of pentylenetetrazol on the vascular system.

(Supported by USPHS Grant B-31.)

317a Recherches sur l'Action Adrenalinogene de l'Ion Potassium chez Differentes Especes Animales. R. Hazard (France).

Les premières expériences faites sur le chien (chloralosé)[1] ont montré la nécessité de réaliser en un temps donné une certaine concentration du sel de potassium dans le sang afférent de la surrénale. L'injection dans la saphène de 0·015 g de KCl par kg faite avec quelques précautions est en

général démonstrative. Pour éviter les dangers pour le coeur de la voie veineuse, on peut faire l'injection dans l'artère rénale à contre-courant, l'aorte abdominale étant temporairement pincée.

Les effets produits (pression, coeur, vaisseaux, intestin, glycémie) sont identiques à ceux de l'adrénaline; ils sont supprimés par surrénalectomie double.

L'action hypertensive est augmentée par la cocaïne et par la spartéine (action postganglionnaire); inversée par yohimbine elle peut reparaître partiellement après spartéine (désinversion).

Chez le chat (chloralosé) l'injection dans la saphène de 0·015 g KCl par kg produit également de l'hypertension que l'yohimbine inverse. Chez le lapin (uréthannisé), même par injection de KCl par l'artère rénale dans l'aorte abdominale, l'hypertension est inconstante et généralement faible. Chez un autre herbivore par contre, le cobaye (uréthannisé) 0·015 g KCl par kg injecté même par la jugulaire produit après une hypotension plus ou moins marquée une nette hypertension. Des adrénalinoverseurs (yohimbine, tolazoline) accentuent la phase hypotensive; ils diminuent ou suppriment la phase hypertensive. Le rat anesthésie au chloral réagit au KCl comme le cobaye.

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1. Hazard, R. (1933).

317b Experiments on the Adrenaline-Releasing Action of Potassium Ion in Different Species. R. Hazard (France).

The intravenous injection of KCl in the dog, anaesthetized with chloralose leads to a release of catecholamine from the adrenal glands (R. Hazard, 1933). The first experiments showed that it was necessary to reach a certain potassium concentration in the afferent blood of the adrenal glands. A somewhat careful injection of 15 mg/kg of KCl into the saphenous vein is generally demonstrative. To avoid the intravenous route, dangerous to the heart, the KCl can also be given by retrograde injection into the renal artery, or into the temporarily clamped abdominal aorta.

The effects produced on the blood pressure, heart, vessels, intestine and blood sugar are identical to those produced by adrenaline; they are abolished by bilateral adrenalectomy.

The hypertensive action is enhanced by cocaine and sparteine (postganglionic action); it is reversed by yohimbine. After yohimbine the hypertensive action can be partially restored by giving sparteine (dis-reversal).

In the cat, anaesthetized with chloralose, an intravenous injection of KCl (15 mg/kg) also produces a hypertension which is reversed by yohimbine.

In the rabbit anaesthetized with urethane, however, even injected into the renal artery or into the abdominal aorta the KCl only causes a variable and small hypertension.

In another herbivore, the guinea-pig, anaesthetized with urethane, KCl (15 mg/kg into the jugularis vein), on the contrary produces a fall in blood pressure which is superseded by a rise. The adrenaline-reversing drugs, yohimbine or tolazoline, enhance the hypotensive phase and reduce or abolish the hypertensive phase.

The rat, anaesthetized with chloral, behaves like the guinea-pig.

318 The Precordial Electrocardiogram during Exercise. J. S. LaDue (U.S.A.).

A new lead and electrode system will be described which permits the recording of 4 precordial leads of the electrocardiogram during mild, moderate and severe exercise. The tracings have steady baselines, are virtually free of muscle noise and permit accurate measurement of amplitude and duration of the P, Q, R, S, ST, T and U waves. The precordial leads obtained are nearly identical with leads V_6, V_8, V_5, V_7 as recorded with the Wilson central terminal.

Results of the electrocardiogram during exercise will be shown in angina pectoris, confusing chest pain and discomfort due to non-cardiac aetiology, some other types of heart disease and the effect of MOA inhibitors and nitroglycerine upon the development and disappearance of abnormal changes.

A comparison of the electrocardiographic changes during and post exercise has disclosed abnormalities not uncovered by the routine Master's single or double 2-step test. This procedure has proved safe since exercise can be stopped when necessary at the onset of pain or the first sign of ischaemia or arrhythmia. Actual demonstrations of the precordial electrocardiogram during exercise will be given at stated intervals.

The normal variations for the electrocardiogram during exercise will be presented together with studies on patients with angina pectoris and healed myocardial infarction.

319 Effect of Cardioactive Steroids on Unipolar Electrocardiograms of Isolated Hearts of Mammals. V. M. Venturi (Italy).

The changes occurring in the duration of electrocardiographic phenomena, after treatment with k-strophantoside and other cardio-active drugs, have been studied recording unipolar electrograms from isolated rabbit hearts.

The RR period is lengthened at low concentrations ($5{\cdot}7 \times 10^{-8}$ M); it is shortened at high concentrations ($2{\cdot}3 \times 10^{-6}$ M). The increase of PQc values is statistically significant only at the highest concentration. The values of both QT and QTc are decreased at all concentrations, the ST interval

being progressively shortened to reach a fusion between the QRS complex and the T wave; biphasism is observed in a preterminal phase, before the onset of fibrillation.

The shortening of QT or QTc values can occur also with treatment with other drugs. However, in the case of drugs causing bradycardia, a reduction of QTc is primarily dependent upon the lengthening of RR, therefore it is not confirmed by a reduction of QT. In the case of drugs causing tachycardia, a shortening of QT is not accompanied by a shortening of QTc. So far, only in the case of cardio-active steroids a QT shortening has been found parallel to a shortening of QTc. This phenomenon seems to be the most constant electrocardiographic response to experimental treatment with cardio-active steroids.

320 Bradyrhythmic Properties of Adenosine and Related Nucleosides. E. T. Angelakos and P. M. Glassman (U.S.A.).

The following compounds were studied in the anaesthetized dog for their acute cardiovascular effects when injected intravenously in doses ranging from 2–25 mg/kg: adenine, adenosine, guanine, guanosine, inosine, cytidine, thymidine, and uridine. With the sole exception of adenosine none of the other compounds listed exhibited any cardiovascular effects. Quantitative dose–response determinations revealed that adenosine is more active as a hypotensive with negative chronotropic (bradyrhythmic) and negative inotropic properties following in that order. None of the adenosine effects were blocked by atropine (even with large doses) and therefore cannot be classified as cholinergic in spite of their similarity to the cardiovasular effects of acetylcholine. Electrocardiographic studies indicated that the bradyrhythmic effect of adenosine is directly on the SA node. Since neither adenine nor any of the related nucleosides were active, it appears that these effects of adenosine are dependent upon the presence of the pentose as well as of the specific purine. These results obtained with the mammalian heart *in vivo* are at some variance with findings on isolated amphibian hearts.

321 Effects of Several Hormones on Erythropoietin Formation and/or Release. J. W. Fisher, J. J. Crook and N. P. Sanzari (U.S.A.).

A number of hormones, some of which are clinically useful, were assayed for erythropoietic activity in male Sprague–Dawley hypophysectomized rats. The compounds tested were injected subcutaneously daily for 2 days. On the third day, a tracer dose of 1 μc ^{59}Fe citrate was given i.v., and 16 hr later blood was removed by cardiac aspiration, the radioactivity counted and per cent ^{59}Fe incorporation into red cells determined. Erythropoiten is a factor found in plasma that, when given to test animals, will stimulate incorporation of ^{59}Fe into red cells or reticulocyte formation, or will increase the size of the red cell mass. The compounds tested and dosage administered per kg were: growth hormone (4 mg); hydrocortisone acetate (4 mg); TSH (4 mg);5:3′ triiodothyronine (700 μg); 11-dehydrocorticosterone (4 mg); angiotensin (100 μg); ACTH (25 units); cobalt-chloride (83 μmg); adrenocortical steroid extract (0·5 ml); and a combination of 3:5:3′ triiodothyronine (16 μg), growth hormone (4 mg) and cortisone acetate (5 mg). The substances stimulating significantly ($P < ·05$) more ^{59}Fe incorporation in red cells than saline injected controls (7·4 ± 2·5%) were: 3:5:3′ triiodothyronine (25·0 ± 7·0%), angiotensin (13·5 ± 3·6%), ACTH (31·27 ± 8·4%), cobalt chloride (27·0 ± 4·4%), adrenocortical extract (12·2 ± 8·6%) and TSH 10·0 ± 3·5%). The kidney is probably the major site of formation of erythropoietin. It is not certain if the compounds tested in this study result in the release of kidney erythropoietin or stimulate the bone marrow directly. Further work is in progress to clarify this point by testing these compounds in the bilaterally nephrectomized rat.

(Supported by USPHS Grant A-2973 C1.)

322 Concerning the Antagonism of Some Central Depressants and Indirect Anticoagulants. P. Hrdina and V. Kovalčík (Czechoslovakia).

The interaction of ethyl biscoumacetate (Pelentan), and phenindione with chlorpromazine, phenobarbital and morphine were investigated on Quick's one stage "prothrombin time" test. The central depressants were administrated for two days, the anticoagulant on the second day. Quick's test was performed every 12 hr for a period of 48 hr. Rats, rabbits and guinea pigs were used.

Chlorpomazine (12 mg/kg) caused no changes whatever. High doses of phenobarbital inhibited significantly the effect of ethyl biscoumacetate, but not of phenindione. Morphine, which itself did not affect coagulation, significantly activated the effect of ethyl biscoumacetate, but lowered the effect of phenindione in rats. The results are discussed.

323 Methods of Discovering Thrombotic and Antithrombotic Substances. Discovery of a New Type of Antithrombotic Effect of Monoamine Oxidase (MAO) inhibitors. T. Shimamoto (Japan).

Today, there is no antithrombotic substance capable of preventing thrombosis without inhibiting extravascular clotting. This is required in the fight against thrombosis.

Almost such an effect was discovered by the authors with MAO inhibitors. The direct traumatization of the internal surface of blood vessels of rabbit's ears by a thread-crushing method using a manometer induced occlusive thrombosis in 15 of

32 control rabbits but in only one of 32 rabbits pretreated with 5 mg/kg of nialamide orally. Of 135 arteriosclerotic patients taking 50–75 mg of nialamide daily, none have exhibited apparent thrombosis during the past one and half years or appreciable side effects including a bleeding tendency.

The principal methods used to find thrombotic substances and antithrombotic substances were microscopic and cinematographic observations on blood vessels of rabbits' mesentery *in situ*, Sandison–Clark's technique, Olef's platelet counting and Lee–White's time determination.

Through these methods adrenaline (0·1 μg/kg i.v.), fatty substances including cholesterol (1 g/kg orally), high molecular substances and cigarette (0·015 g/kg) smoking were found to induce sticking of platelets and leucocytes to the vascular endothelium, clumping and white thrombus-formation rabbits and immediate decrease in the platelet count and shortening of Lee–White's time in men and rabbits. Such reactions represent the phylogenetically old mechanisms to stop bleeding. Adhesion and clumping of platelets and leucocytes to damaged endothelium may prevent washing away of soluble clotting factors in the blood stream and keep them concentrated in this part. MAO inhibitors block these reactions without inhibiting further the process of blood coagulation *in vivo* and also *in vitro*.

324 Experience from Trials with a Thrombolytic Enzyme. B. Mogensen, V. Larsen and O. Storm (Denmark).

In the therapy of thrombo-embolic diseases, the conventional, palliative anti-coagulant treatment has during recent years partly been replaced by attempts to establish a more rational therapy with a view to thrombolysis. For this purpose, activators of the fibrinolytic system, combinations of activators and thrombolytic enzymes (plasmin) and plasmin only, have been employed. Experience from animal experiments (Princeton 1960) in which considerable plasmin doses were given has shown that it is possible to dissolve preformed thrombi, but also that this method of administration may give rise to side effects such as shock, temporary hypercoagulability and afibrinogenaemia. Recent studies have shown that it is possible to reduce greatly the occurrence of these side effects by employing balanced doses of highly-purified plasmin; furthermore, the combined administration of plasmin and heparin makes it possible to reduce considerably the doses of the former, whereby the occurrence of side effects is avoided without affecting the thrombolysis.

325 Cellular Study of Drug Alteration of Prothrombin Synthesis. M. I. Barnhart (U.S.A.).

Dynamic aspects of prothrombin synthesis were studied in dogs following administration of drugs which alter the circulating prothrombin concentration. The fluorescent antibody technique provided information on the cellular function of coumadin and vitamin K_1. Fluorescent anti-dog prothrombin was used to mark those cells containing adequate amounts of prothrombin. Blood samples were taken concurrent with liver biopsies to determine the status of circulating coagulation factors. In the normal animal, only a few liver parenchymal cells contained enough prothrombin to exhibit specific fluorescence. Our studies suggest that normally there is asynchronous cyclic production of prothrombin. When circulating prothrombin was drastically reduced by i.v. administration of coumadin, specific liver cell fluorescence decreased as the prothrombin deficiency progressed. This study indicated that the mechanism of action of coumadin was to block biosynthesis of prothrombin rather than promote its storage. Vitamin K_1 administration to such dogs re-established synthesis of prothrombin. A brief lag period occurred during which precursors were mobilized and prothrombin formed in sufficient amounts to be detected by the fluorescent antibody. Within three hours essentially all liver parenchymal cells exhibited intense specific fluorescence although the circulating prothrombin remained low. The cellular fluorescence declined later as the circulating prothrombin returned to normal. Prothrombin synthesis in the normal dog also was synchronized by vitamin K_1, but there was only limited release of prothrombin to the blood. Some regulatory mechanism seems to monitor the circulating prothrombin and to maintain it within limits by promoting either storage, synthesis or release of prothrombin from liver parenchymal cells.

(Aided by Michigan Heart Association and USPHS Grant H-4712.)

326 Clinical Factors Influencing the Redox-potential in the Blood. J. Rehn (Germany).

Humoral factors play an important role in shock. Increased proteolysis leads to a locally, as well as generally, increased release of biologically and pharmacologically active peptides and amines. For this phenomenon the redox-potential (RP) very important. This can be proved by clinical and experimental examinations. In large groups of patients, the significance of the RP in the pre- and post-operative courses of different diseases was examined. The reaction produced by medication with redox-active preparations allows conclusions concerning the general starting condition of the patient. The central position of vitamin C for RP changes has been proven, and strengthened by many new experimental results; just recently, further connexions between copper (ceruloplasmin)—iron metabolism were detected. These examinations will be briefly described.

327 The Oxidation-Reduction Potential of Blood under Different Pharmacological and Pathophysiological Conditions. E. ZIEGLER (Switzerland).

A new method for measurement of the redox potential (RP) of blood is described and illustrated by means of the methylene blue reaction. The RP of blood is determined by a kinetic oxidation-reduction equilibrium (ascorbic acid system) and gives an indication of the reduction intensity of the tissues. Physiological and pharmacological factors produce characteristic changes in RP. The same is true of pathophysiological states which are associated with hypoxaemia or enzyme blockade (haemorrhagic shock, CO and CN poisoning). The liver plays a primary role in these conditions. These findings which were mainly observed *in vivo* in animal experiments, have been confirmed by *in vitro* studies in the clinic. Moreover, in man the administration of redox-active substances has revealed close relationships between RP and aspecific regulatory processes of the organism (Rehn). The potential while mainly determined by the ascorbic acid system is also influenced by Cu and Fe catalysts. These and the ascorbic acid system constitute the above-mentioned dynamic equilibrium. Evidence for this has also been obtained in studies with respiratory inhibitors *in vitro*.

328 The Chelation of Ca^{2+} and Mg^{2+} by EDTA at Different Acidities. P. GEORGE (U.S.A.).

The therapeutic action of EDTA in the treatment of cardiovascular and other diseases depends in some way on its ability to chelate metal ions. To appreciate more fully the nature of the reaction that occurs in the physiological pH range, it is necessary to formulate exactly how acidity affects the chelation process. The effect is complicated because the ligand (EDTA), the metal ion, and the complex formed, all take part in acid–base equilibria.

Denoting the completely protonated species of EDTA by H_6L^{2+}, the formation of the calcium complex occurs predominantly via the following reactions in the pH ranges indicated:

strong acid: $Ca^{2+} + H_6L^{2+} \rightleftharpoons CaHL^{1-} + 5H^+$
pH 4–5: $Ca^{2+} + H_2L^{2-} \rightleftharpoons CaL^{2-} + 2H^+$
pH 7–8: $Ca^{2+} + HL^{3-} \rightleftharpoons CaL^{2-} + H^+$
pH ≈ 11: $Ca^{2+} + L^{4-} \rightleftharpoons CaL^{2-}$
pH > 12: $Ca(OH)_2 + 2H^+ + L^{4-} \rightleftharpoons CaL^{2-} + 2H_2O$.

There are marked changes in the participation of hydrogen ion, both in number and in its role as product or reactant. The "observed" stability constant, K_{obs} = [Complex] / [Uncombined Ligand] × [Uncomplexed Calcium] thus varies with pH in a complicated manner. The corresponding standard free energy change, $\Delta F_{obs} = -RT\ln K_{obs}$, has been evaluated from data in the literature for both Ca^{2+} and Mg^{2+} complexes over the entire pH range 0–14. A term involving $[H^+]$ contributes very substantially to this observed standard free energy change, and its origin and significance will be briefly discussed.

(This work is being conducted under a grant from the John A. Hartford Foundation.)

329 The Action of Morphine on Impulse Propagation in Nerve Fibres. A. B. CAIRNIE and H. W. KOSTERLITZ (United Kingdom).

Morphine reduces the response of certain, but not all, autonomic neuro-effectors to stimulation of their pre- or post-ganglionic nerves.[1,2] For the analysis of this effect it is important to know whether morphine has an action on impulse propagation in nerve fibres.

Threshold stimulus and conduction velocity were measured, before and after intravenous injection of morphine (1–5 mg./kg.), in A-B and C groups of fibres of the rabbit vagus nerve and in α and C groups of the cat saphenous nerve. Experiments were also performed on the α and δ groups of the saphenous nerve to find out whether there is an effect of morphine on the recovery of excitability and on the height of the maximal action potential. Morphine had no effect on any of these parameters.

1. KOSTERLITZ, H. W. and TAYLOR, D. W. (1959), *Brit. J. Pharmacol.*, **14,** 209.
2. KOSTERLITZ, H. W. and TAYLOR, D. W. (1959), *J. Physiol.*, **149,** 59P.

330 Mechanism of Peripheral Nerve Block. G. A. CONDOURIS (U.S.A.).

It has been shown that cocaine and other local anaesthetic agents block conduction in isolated bullfrog peripheral nerves by a mechanism which involves a competitive antagonism between the anaesthetic and the sodium associated with nerve function.[1] The question of the general applicability of this hypothesis to nerve block in mammals remained, however, since amphibian nerves could conceivably have a sodium-dependent, anaesthetic-sensitive physiological mechanism not found in mammalian nerves. An exploration of the interrelationships between sodium and cocaine on nerve conduction was made on isolated sciatic-tibial trunks from rats and, as with amphibian nerves, a remarkable dependence was found between the degree of conduction block by cocaine and the relative concentrations of sodium and the anaesthetic in the extra-fibrillar environment. An increase in the concentration of either substance necessitated a proportional increase in the concentration of the other if a given degree of block was to be maintained. While the sodium–cocaine interactions in the rat nerve were qualitatively similar to those described for frog nerves, several quantitative differences were found which serve to re-enforce

the argument that cocaine interferes with the neurotropic action of sodium and which also help to explain why mammalian nerves show a greater susceptibility to the action of local anaesthetics than do amphibian nerves. The results found for rat nerve thus confirm the findings on frog nerve and make it appear that the competitive antagonism hypothesis has general applicability to peripheral nerve.

1. Condouris, G. A. (1961), *J. Pharmacol.*, **131,** 243.

331 Modes of Action of Tris(hydroxymethyl) amino methane (THAM). G. G. Nahas, S. J. Dos, J. Strauss and M. H. Holmdahl (U.S.A.).

THAM is an organic proton acceptor of low toxicity, very water soluble, which binds (H^+) resulting from respiratory or metabolic acidosis. At 37°C the p*K* of a 0·3 M solution (isosmolar with body fluids) is 7·84; at normal body pH (7·40) THAM is 70 per cent ionized; it binds on plasma protein. It is not readily absorbed from the gastro-intestinal tract and must be administered intravenously. Studies with ^{14}C tagged material indicate that it distributes within 4 hr into 65–80 per cent of body water. THAM could, therefore, act as an intracellular buffer. In addition to its buffering property, it is a diuretic, it depresses ventilation (through a specific or a pH effect on the respiratory centres), and in large doses (0·5–1 g/kg) produces hypoglycaemia. Solutions of 0·3–0·6 M THAM increase diuresis by 4 to 10-fold with production of an alkaline urine (7·34–7·68) and an increased elimination of Na^+, K^+, Cl^-, and HCO^-_3. Up to 60 per cent of the filtered load of HCO^-_3 may be eliminated indicating that THAM, in addition to its osmotic activity, could act on the tubular cells. THAM also increases by 5-fold salicylate elimination by the kidney. Its hypoglycaemic action which is minimized in pancreatectomized dogs may be due to its protein binding, which could compete with insulin binding and, therefore, increase the action of the latter. Two possible therapeutic applications of THAM are in insulin-resistant diabetic acidosis and in salicylate poisoning.

332 The Mode of Action of Buffers in Modifying the Effects of Oxygen Toxicity in Mice. C. Sanger and G. G. Nahas (U.S.A.).

The intraperitoneal injection of 0·3 M tris (hydroxymethyl) amino methane (THAM), or the repeated intraperitoneal injections of 0·3 M $NaHCO_3$ significantly increases the number of 24-hr survivors in mice exposed to 100 per cent oxygen at 30, 42 and 55 p.s.i. and lengthens the latent period before the onset of convulsions.

The administration of 5 per cent dextrose, or of sodium bromide in various doses also increases the preconvulsion time, but does not significantly alter the number of mice surviving after exposure.

The fact that the effects of oxygen toxicity in mice may be modified by altering the buffering capacity of the body fluids suggests that disturbances of CO_2 transport and storage may be of importance in the causation of these effects. The increase in the number of survivors indicates that this buffering action may be associated with some of the fundamental mechanisms involved.

333 The Mode of Action of a New Intravenous Anaesthetic, G29505. M. Swerdlow (United Kingdom).

A new and interesting intravenous anaesthetic (G29505) has been administered to over 400 patients. The mode of onset of sleep and emergence from anaesthesia have been studied, as well as the duration of action. In addition, the effects of this drug on the respiration, cardiovascular system, etc. have been noted. A series of patients undergoing similar operations under comparable conditions have been anaesthetized with thiopentone (Pentothal) and the results obtained with G29505 are compared with those obtained with the patients who received thiopentone. Some pharmacological inferences are drawn from this comparison.

334 Oxygenation through Absorption from the Intestinal Tract. H. F. Häubler and S. Fahmy (Austria).

In anaesthetized cats and dogs kept under hypoxic conditions by letting the animal breathe a mixture of 5 per cent oxygen in nitrogen, oxygen was supplied either by inflation into the small intestine or by perfusion through the colon. During this application of oxygen an increase of the O_2-content of the blood could be observed. In other experiments in cats, perfusion of 3 per cent (w/v) solutions of H_2O_2 through the colon also produced an increase of the O_2-content of the blood. By these means a lack of pulmonary respiration could be compensated.

It was found that by either method sufficient amounts of oxygen were absorbed by the blood to keep the oxygen content at normal levels for a period of about 15 min. It is concluded that intestinal application of oxygen seems to be a way which might be of clinical importance to overcome short periods of failure in pulmonary respiration.

335a Das Verhalten der Sauerstoffaufnahme und der Körpertemperatur von Meerschweinchen in Urethan-Narkose. M. Maeß und J. Pichotka (Deutschland).

Es ist aus zahlreichen Experimenten bekannt, daß durch Narkose eine Senkung der Körpertemperatur zustande kommt. Im allgemeinen wird angenommen, daß es sich dabei um eine

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zentrale Störung der Temperatur-regulation handelt, insbesondere da bekannt ist, daß in manchen Situationen der Stoffwechsel erhöht sein kann, obwohl die Körpertemperatur gesenkt ist. In 80 Experimenten mit kontinuierlicher Registrierung von Körpertemperatur und Stoffwechselgröße ließ sich zeigen, daß die Stoffwechselgröße beim Meerschweinchen nach der Injektion von Urethandosen zwischen 0·2 und 1·5 g/kg im allgemeinen innerhalb 10 min auf einen Minimalwert abgesunken ist. In einzelnen Fällen ist der Minimalstoffwechsel 3 min nach der Injektion erreicht. Zum Zeitpunkt des Minimalstoffwechsels ist die Rektaltemperatur oft kaum oder erst unwesentlich gesenkt. Die Senkung der Rektaltemperatur, die dieser Stoffwechselsenkung korrespondiert, müßte nach der üblichen Erfahrung in der Größenordnung von Stunden erfolgem. Der zeitliche Verlauf der beiden Größen nebeneinander zeigt, daß im Bereich der geringen Dosen der Abfall der Rektaltemperatur von einem Wiederanstieg des zunächst stark erniedrigten Stoffwechsels begleitet ist. Dabei können mit fallender Temperatur kontinuierlich ansteigend Stoffwechselgrößen erreicht werden, die über dem Ausgangswert liegen, entsprechend der eingangs berichteten Beobachtung. Mit steigender Dosis wird der Wiederanstieg der erniedrigten Stoffwechselgröße mit der fallenden Rektaltemperatur zunehmend geringer und bleibt bei den höheren Dosen schließlich ganz aus. Die Prüfung der chemischen Temperaturregulation durch Standardabkühlungen zeigt, daß der Stoffwechselanstieg für die gleiche äussere Abkühlungsgröße mit steigender Narkosedosis fällt. Bei Dosen oberhalb 1 g/kg kommt es zum völligen Ausbleiben der Stoffwechselsteigerung bei Abkühlung; Körpertemperatur und Stoffwechselgröße bewegen sich nicht gegensinnig, sondern gleichsinnig. Nach dem zeitlichen Verlauf und nach der Dosisabhéngigkeit der Störungen muß man schließen, daß der primäre Angriffspunkt für die Störung der Temperaturregulation in der Erniedrigung der Stoffwechselgröße liegt.

335b The Course of Oxygen Uptake and Body Temperature in Guinea-Pigs under Urethane Narcosis. M. Maess and J. Pichotka (Germany).

It is known from numerous experiments that narcosis results in a fall in body temperature. In general, it is assumed that this is a matter of central disturbance in temperature regulation, the more so since it is known that in some situation metabolism may be raised although the body temperature has dropped. In 80 experiments with continuous recording of body temperature and metabolic values, it has been shown that the level of metabolism in guinea-pigs after injection of between 0·2 and 1·5 g/kg urethane as a rule fell within 10 min to the minimum value. In individual cases, the metabolic minimum was reached within 3 min of injection. At the time of the lowest metabolism, the rectal temperature had hardly fallen or started to fall slightly. The fall in rectal temperature corresponding to this lowered metabolism should according to usual experience be a matter of hours. The time course of both values shows that, in the low-dose range, the drop in rectal temperature accompanied by a renewed rise in the initially heavily-reduced metabolism. As the temperature goes on falling, continuously rising values of metabolism may be reached, exceeding the initial value corresponding to the first observation. With increase in dosage, the renewed rise in the lowered metabolic level with falling rectal temperature becomes ever smaller and, finally, at higher doses, is completely absent. Testing of chemical temperature regulation by standard cooling showed that the rise in metabolism for the same external cooling falls with increasing dose of anaesthetic. At doses above 1g/kg there is no rise at all in metabolism for the same external cooling falls with increasing dose of anaesthetic. At doses above 1 g/kg there is no rise at all in metabolism on cooling; the body temperature and metabolic levels move not in opposite but the same direction. From the time course and dose dependence of the disturbances it must be concluded that the primary point of attack which causes disturbances in temperature regulation lies in lowering of the level of metabolism.

336 Some Oxdiazolones as Anti-pyretic, Analgesic and Anti-inflammatory Agents. A. E. W. Smith (Norway).

Many attempts have been made to replace acetylsalicylic acid by other more active and less toxic agents. Thus γ-resorcylic acid has been tested in the treatment of rheumatic fever by Reid *et al.*[1] who found that its effect on temperature, pulse and respiration rates was not quite so spectacular as that produced by salicylates. Profuse sweating, which often occurs with salicylates, did not occur with γ-resorcylic acid. Toxic signs (emotional instability, mental confusion, muscular weakness, etc.) were, however, observed. There was absence of tinnitus and deafness in patients treated with γ-resorcylic. Thus, toxicity and pharmacological activity do not necessarily run parallel in this type of compound, which suggests further research is necessary.

A number of oxdiazolones have been found to be active anti-inflammatory, analgesic and anti-pyretic compounds showing a low toxicity.

The pharmacology and toxicity of these compounds have been investigated and compared under under standard conditions with aspirin.

1. Reid, J., Watson, R. D., Cochrane, J. B. and Sproull, D. H. (1951), *Brit. med. J.*, **2**, 321.

337 Study on Electrolyte Absorption from Inflamed Connective Tissue by means of Radioactive Isotope. L. Szporny (Hungary).

Radioactive phosphate was injected into the plantar surface of the paw of the rat, and the activity found in the paw was measured for 21 min by means of an end-window G.M. tube. In order to produce inflammation, 1–100 μg serotonin, 1 mg dextran, 2 mg formalin, 100 μg histamine and 100 I.U. hyaluronidase were injected into the paw. The oedema produced was measured by the volumetric method described by us. It has been found that the absorption of the PO_4 was not influenced by histamine formalin or 1 μg serotonin when compared with the untreated paw, dextran and 10 μf of serotonin decreased the rate of absorption to a small extent, while 100 μg serotonin show a potent decreasing action. The dispersion of the PO_4 was slightly but significantly accelerated by hyaluronidase. During the period of examination, oedema of 127–470 mm^3 was produced by the action of the various compounds, the rate of swelling being disproportionate to its effect on the absorption of the compounds.

Pretreatment of the animals with 150 mg/kg phenylbutazone parenterally, or 10 mg/kg prednisolone did not influence the absorption of phosphorous from untreated paws. Pretreatment with an antiphlogistic in addition to its action on the development of the oedema did not influence electrolyte absorption.

The results obtained show that electrolyte absorption is due to a quite different mechanism from that of the development and blocking of oedema. The absorption inhibiting action of some phlogistics is probably the result of their vasoconstricting effect. The accelerating action of hyaluronidase is due to its increasing effect on the permeability of the connective tissue basic substance.

338 Studies on the Mechanism of the Anti-Appetite Action of Phenmetrazine. A. Engelhardt (Germany).

After subcutaneous injection, both the optically active stereoisomers of phenmetrazine are about equally effective in inhibiting food consumption in rats. After oral administration the levoisomer is significantly more effective. On the other hand, in respect of central nervous stimulation the dextroisomer is about four times more active than levorotatory phenmetrazine, irrespective of whether administration is subcutaneous or oral. The secretion of gastric juice and hydrochloric acid is inhibited in Shay rats by a dose of 10–20 mg/kg, which influences the food consumption, or by perfusing the stomach with a concentration of 10^{-4} mg/ml. With the same dosage the gastric motility is markedly reduced, as evidenced by the prolonged emptying time of the stomach after a contrast meal followed by X-ray examination. In this respect also L-phenmetrazine is more active than the dextroisomer. On isolated vagus-stomach preparations a concentration of 10^{-5} g/ml inhibits the motor response of an electric stimulus of the vagus nerve almost completely, whereas the histamine and acetylcholine contraction is only slightly affected. In earlier experiments with subcutaneous injections of ^{14}C-labelled phenmetrazine,[1] the radioactivity has been found to be concentrated in the stomach wall and "secreted" into the gastric contents. The experimental results lead to the conclusion that in the anti-appetite effect of phenmetrazine there is involved a mechanism acting peripherally on motility and secretion of the stomach. There exists some evidence that a similar mechanism contributes to the action of other anti-appetite drugs.

1. Engelhardt *et al.* (1958), *Arch. Exp. Path. Pharmak.*, **235**, 10.

339 A Contribution to the Knowledge of the Physiology of the Digestive Tract of Fish by means of Diascopy with Special Application of a Contrast Medium (Barium Sulphate). T. Švob (Yugoslavia).

In some previous researches we presented by our modified method the radiography of the digestive tract of some species of Actynopterygii and Selachii. In these researches, in which our method was the application of contrast (suspension of barium sulphate) by means of oral and anal sounding of live fish, we were able to establish numerous morphological and some physiological properties of the digestive tract of these animals. During the X-ray study of the physiology of the digestive tract we encountered many difficulties because these poikilothermal organisms would not take food spontaneously under given circumstances, and there was often no adequate motor response to the forced introduction of barium suspension.

In our work, presented here, we avoided these negative effects of the intrusion of the contrast medium. At the same time, we made a further contribution to research into the digestion of fish, as we were able to watch the initial dissolution of the food taken into their digestive tract. We made three specific kinds of capsule: those with a coating of an albuminous substance (cattle serosa), those with a coating of carbohydrate (flour), and those with a coating of fat (bovine fats). The centre of each of these capsules consisted of barium sulphate. After the oral intrusion of these capsules into the intestinal system of the fish the dissolution of the coating and the liberation of the contrast medium occurred in specific parts of their digestive tract at specific intervals. This we were able to establish by means of diascopy. Thus we can indicate the regions in the digestive tract of the tested species where digestion of the given food begins.

340 Kinetics of some Biligraphic and Urographic Contrast Media. E. Beccari and F. Bonati (Italy).

Six iodinated contrast media (iodopyracet; diatrizoate; iodipamide; iopanoic acid; 3-acetylamino-2:4:6-triiodo-phenoxyacetic cid; 3-acetylamino-2:4:6-triiodo-phenoxy-α-caprocic acid) were given to rabbits by intravenous injections and their blood levels, as well as biliary and urinary excretion, have been followed up to 3 hr after administration.

Estimations were performed every minute during the first 10 min and every 10 min afterwards. The most characteristic patterns were observed very early after the injection: the blood concentration curves, plotted on semilogarithmic paper, showed four components, for which the intercept for zero time and the slope have been determined.

According to the compartment theory, such patterns may be accounted for either by a four nodes net with recycle, or by two or three basic compartments in which linear and partially non-linear processes occur. Attempts to decide which model is more reliable in fitting the observed data have been made by means of a Donner analogue computer and by a change in the experimental conditions.

In all cases the values obtained for biligraphic media are in quite a different range from those relating to urographic ones.

In the later stages of blood distribution, as well as in biliary and urinary elimination curves, only one component seems to be involved.

341 Mode of Action of Drugs at the Choledochoduodenal Junction. A. Crema and F. Bertè (Italy).

There are very close connexions between the duodenum and the terminal part of the choledochus, but they are not yet well known from an anatomical and physiological point of view. It is very likely that the supposed action of some drugs on the tonus and rhythmic movements of Oddi's sphincter is at least the result of interference at the duodenum and the choledochoduodenal junction. In order to study separately the action on the different organs, we used many methods in various animals: a strip of sphincter, the isolated but entire sphincter, the duodenum isolated with the lower choledochus keeping intact the proper vessel and the whole animal in which the drugs were injected directly into the artery which supplies Oddi's sphincter and the neighbouring duodenum. By these methods we studied the action of many drugs such as sympathomimetics and parasympathomimetics, analgesics, smooth muscle relaxants, etc.

342 The Mammotropic Effect of Ataractic Drugs. F. G. Sulman (Israel).

In 1955 Winnik and Tennenbaum[1] showed that chlorpromazine may produce galactorrhoea in human patients. Further studies in animals by Sulman and Winnik in 1956[2] elaborated the mechanism of the mammotropic effect. It is based on suppression of the hypothalamus and the secondary feed-back reactions of the pituitary and the peripheral glands with endocrine secretion.

The hypothalamus, through its portal veins as well as through its cholinergic and adrenergic fibres, regulates the anterior and posterior lobes of the pituitary gland. This effect balances itself in accordance with the push and pull principle so far as the following tropins are concerned:

FSH & LH —— oestrone, progesterone, testosterone;
ACTH —— corticoid steroids;
TSH —— thyroxine, tri-iodothyronine;
STH —— glucagon.

These tropins should be called "balanced tropins" since they are permanently kept in balance by the peripheral hormones, which they produce. On the other hand, there exist "unbalanced tropins" and posterior lobe hormones, such as LTH, STH, MSH & ADH. These are not checked by any secondary hormones and therefore an overshoot and rebound phenomenon is possible, when their equilibrium is disturbed by hypothalamic depression. With regard to chlorpromazine and reserpine we have established the sequence of processes shown in Table 1.

Table 1
Hormonal effect of protracted treatment with hypothalamus depressants

Stage	Duration	Effect
1	First week	Hypothalamus depressed Pituitary depressed
2	Second week	Hypothalamus depressed Peripheral glands depressed
3	Third week	Hypothalamus depressed Hormonal balance restored
4	Fourth week	Hypothalamus depressed 3 Possibilities of balance: (1) Equilibrium of balanced tropins (2) Depression of balanced tropins (3) Rebound of unbalanced tropins LTH, MSH, ADH

Recently, the nipple test in the male rat has proved to be a reliable guide for the ranking of the mammotropic potency of ataractic drugs. The histological picture clearly allows differentiation between an oestrogenic, progestogenic and mammotropic pattern typical for the different ataractic

drugs. Thus the rat nipple test may serve for the prediction of lactogenic side-effects in human effects.

1. Winnik, H. Z. and Tennenbaum, L. (1955), *Pr. méd.*, **63,** 1092.
2. Sulman, F. G. and Winnik, H. Z. (1956), *Lancet*, **1,** 161; (1956), *Nature (Lond.)*, **178,** 365.

343 Mode of Action of 5-HT and Iproniazid on Pregnancy. M. Botros, D. Lindsay, E. Poulson and J. M. Robson (United Kingdom).

It has been shown previously that 5-hydroxytryptamine (5-HT) and certain amine oxidase inhibitors, will interrupt pregnancy in mice. The latter drugs are only effective during the first half of pregnancy, whereas 5-HT is effective at all stages.[1] Similar effects can be produced on the experimental decidual reaction in mice showing that the action is not on the foetus,[2] as is the case with many nucleotoxic drugs,[3] but on the placenta. Some of these effects on the placenta can be prevented by the simultaneous administration of progesterone and, to a lesser extent, of luteotrophin. It is suggested that these drugs act at the level of the brain, interfering with the mechanism responsible for the maintenance of the corpus luteum. This is in keeping with the finding that these drugs will prevent the development of sexual maturity when given to immature female mice.[4]

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2. Lindsay, D., Poulson, E. and Robson, J. M. (1961), *J. Endocr.*, **22,** v.
3. Robson, J. M. (1959), *Memoirs of the Society for Endocrinology*, **6,** 54.
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344 On the Mechanism of Protection by Pregnancy against Acute Hyperparathyroidism. D. Leiir and M. Krukowski (U.S.A.).

Adult albino rats, injected with 150 U.S.P. units of parathyroid hormone (PTH) twice daily subcutaneously for a maximum period of 3 days, develop pronounced hypercalcaemia and hyperphosphataemia and usually succumb within 3 days with signs of depression, anorexia and respiratory difficulty. Such animals invariably exhibit severe necrosis and calcification of myocardium and kidneys.

In the course of studies on the influence of this acute hyperparathyroid state on gestation and foetal growth, it was found that advanced pregnancy (third week) provided remarkable protection against the toxic sequelae of PTH overdosage. Protection entailed a striking decrease in the severity of nephrocalcinosis and complete prevention of myocardial injury. Hypercalcaemia was milder and more transient and hyperphosphataemia did not occur. Hence, rats near term survived the PTH injection period in good physical condition. Immediately following delivery of the offspring, the protective effect against excess PTH could no longer be elicited.

Limitation of protective activity to the period of most rapid foetal growth and its abrupt abolition following parturition, pointed towards participation of the conceptus in the maternal resistance to excess PTH. Studies designed to distinguish between the possible importance of foetal and placental factors are at present in progress. Preliminary findings would seem to implicate the foetus in the mechanism of protection.

Additional parameters under exploration include gonadotropic, ovarian and adrenal hormones of the mother as well as mineral metabolic alterations with special attention to calcium and inorganic phosphate changes.

(Aided by Grant No. H-8/0 (C10) from the National Heart Institute, U.S. Public Health Service.)

345 Some Observations on the Effect of Oestrogens on the Pituitary-Adrenal System. A. M. Barrett (United Kingdom).

Determination of peripheral plasma corticosterone levels in the rat under conditions approximating to rest demonstrates marked difference between the two sexes. Female values are significantly higher than males and exhibit greater variation. When the phase of oestrus is taken into account, it is found that the highest values occur in met-oestrus and dioestrus and the lowest in pro-oestrus and oestrus. The concentration of corticotrophin in the female adenohypophysis also varies with the phase of oestrus, being highest when the concentration of plasma corticosterone is highest. However, the release of corticotrophin in response to ether excitation is greatest in pro-oestrus and oestrus. In addition, the maximal secretory capacity of the adrenal cortex is greatest when oestrogen secretion is high. The role of oestrogen in the regulation of pituitary adrenocorticotrophic activity will be discussed.

346 Studies on the Intestinal Absorption of Steroid Hormones in the Rat. K. H. Kimbel (Germany).

The low oral activity of testosterone and progesterone derivatives may be explained by insufficient intestinal absorption as well as by inactivation during liver passage. A third possibility, the delayed transport through the lymphatics has further to be taken into consideration.

The ingenious experiments of Schedl have clarified the problem to some extent demonstrating that nearly all steroid hormones are absorbed at a con-

stant percentage independent of absolute concentration and amount.

To study this matter further experiments were undertaken in male albino rats with portal vein fistulas and abdominal lymphatic fistulas according to Bollman. By means of ^{14}C-labelled derivatives of testosterone and progesterone the total amount absorbed from the intestinal lumen could be determined. Furthermore, the concentration in portal vein blood and abdominal lymph, and the total output in the lymph have been estimated. The influence of chemical structure, concentration and different solvents on the absorption kinetics has been studied.

347 The Antigonadotrophic Activity of Some Progestinics Given by Mouth. A. Ercoli and G. Falconi (Italy).

It has already been reported that enol ether resulting from the condensation of Δ^4-3-ketosteroid hormones with suitable alcohols display an enhanced biological activity by mouth in comparison with the parent steroids. This has been shown to be true for enol ethers of some progestational compounds, on the basis of both Clauberg and pregnancy maintenance test.[1, 2]

The same compounds administered orally were then investigated with respect to their ability to influence the release of gonadotrophic hormones. In particular, the interference of given compounds with oestrous cycle, fertility and lactation has been studied in rats. The antigonadotrophic activity in parabiotic rats was also examined.

This research was performed with the cyclopentylenol ethers of progesterone, 17α-acetoxyprogesterone, 6α-methyl-17α-acetoxyprogesterone and 17α-ethynil 19-nortestosterone acetate in comparison with the parent ketones and with injected progesterone.

It has been found that there is no relation between progestational and antigonadotrophic activity.

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2. Falconi, G., Gardi, R., Bruni, G. and Ercoli A., *Endocrinology*, in press.

348 Studies on Mechanisms Controlling Luteotrophic Hormone Release. F. Fraschini, L. Martini, M. Motta and A. Pecile (Italy).

The modes of the response of the pituitary after the exposure to noxious stimuli or the administration of drugs have not been clarified so far. According to some experiments a specific response leading to the release of ACTH could be postulated, whereas according to others a response characterized by the simultaneous release of several pituitary hormones (ACTH, TSH, gonadotrophins, etc.) seems more probable. The purpose of this study was to investigate the influence on the secretion of luteotrophic hormone (LTH) of several pharmacological agents and stresses known to induce ACTH release.

The decidual response in normal-cycling female rats was used as the index of luteotrophic hormone release. Pharmacological agents and stress procedures were applied daily for three days starting from the day of oestrus. The uteri of animals suspected of being pseudopregnant were traumatized by threading one horn, on the day following the last treatment. Autopsy was performed 5 days after traumatization.

Deciduomata were obtained after subcutaneous injections of acetylcholine, adrenaline, histamine, serotonin, salycilate, formalin, insulin and synthetic oxytocin; the injection of several antidiuretic hormone preparations was always ineffective. Positive responses were also obtained after intraperitoneal injections of hypertonic saline solution and after submitting the animals to asphyxia, electric shock and ether anaesthesia.

349 The Effect of Injected Oestrogen on Amino-acid Incorporation into Chick Oviduct. S. M. Kalman and P. M. Opsahl (U.S.A.).

Cell-free fractions of chick oviduct incorporate amino-acids into protein after treatment with oestrogen. Chicks, 5 weeks old, were injected with 1·5 mg stilboesterol or oestradiol 24 hr before sacrifice. The control received no oestrogen. Oviducts were homogenized in incubation medium (without added cofactors and ATP generating system). Cell fractions, prepared by centrifugation, were incubated at 37°C with 2 μM L-[1-^{14}C] leucine (specific activity 6·2 μC/μM) in medium containing 0·25 M sucrose, 0·02 M K phosphate buffer (pH 7·4), 0·025 M KCl, 0·035 M KCO_3, 0·01 M $MgCl_2$ plus 1 μM ATP, 0·25 μM GTP, 10 μM phosphoenolpyruvate, and 0·03 mg phosphoenolpyruvatekinase per ml. When the 5000 × *g* supernate from control tissue was incubated in this medium, 6 counts/min/mg protein were incorporated; 43 counts/min/mg protein were incorporated by the same fraction from oestrogen-treated chicks. In a comparable experiment the control 5000 × *g* fraction incorporated 12 counts/min/mg protein against 52 counts for oestrogen-treated chicks. The 5000 × *g* supernate from control and also from experimental animals was separated into the 105,000 × *g* supernate and the 105,000 × *g* pellet. Combination of the pellet from control with supernate from oestrogen-treated tissue yielded 10 counts/min/mg protein, the pellet from oestrogen-treated chicks combined with supernate from controls yielded 53 counts/min/mg protein. When the two fractions from the same

source were recombined, there were 5 counts/min/mg protein for control tissue, and 55 counts/min/mg protein for oestrogen tissue fractions.

These data indicate that oestrogen treatment enhances ability of subcellular fractions to incorporate amino-acids into protein at a time when oviduct growth *in vivo* is markedly enhanced. This enhanced activity is associated with the pellet obtained by high speed centrifugation, presumably the microsomes.

350 Parathyroid Hormone and the Placental Barrier. M. KRUKOWSKI and D. LEHR (U.S.A.).

Albino rats, when exposed to overdosage of parathyroid hormone (PTH) in the late stages of gestation, exhibited hypercalcaemia, loss in body wt. and moderate degrees of cardio-renal injury. The foetuses of such animals, on the other hand, were unaffected in growth and development and did not exhibit any increase in serum Ca levels. Such elevation could be readily achieved, however, by injection of PTH directly into the foetus.

Control rats in advanced pregnancy were normocalcaemic, whereas their unborn offspring were hypercalcaemic (15–18 mg per cent). After birth, serum levels of the young declined to the normocalcaemic range of the mothers or dropped substantially below it. Excised foetuses breathing spontaneously manifest the same fall. No substantial deviations from the physiological serum Ca changes occurring at birth were noted in litters delivered by acutely hyperparathyroid rats.

The absence of conspicuous interference with foetal development speaks for protection of the conceptus either by the placental barrier or by special resistance of foetal tissues to PTH. Failure to influence foetal serum Ca in the face of high PTH concentrations in the maternal circulation, and the ready production of elevated foetal Ca levels by the introduction of PTH directly into the foetus, would seem to favour the placental barrier as the more probable mechanism of foetal protection against maternal hyperparathyroidism.

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351 Reserpine and Thyroid Function in the Fowl. B. N. PREMACHANDRA (U.S.A.).

Tranquillizers have been suggested for use with poultry because of their stress ameliorating properties, with possible beneficial effect on egg production, growth and feed efficiency. One of the more important of the rauwolfia alkaloids employed has been reserpine, and sporadic reports are not uncommon in the literature indicating its anti-thyroid effect in experimental animals. If this was true of poultry, administration of reserpine would then be contra-indicated for purposes of sustained tranquillization where normal thyroid function is desired. A method for the precise determination of thyroid function in fowls being available,[1] experiments were carried out in New Hampshire chickens, involving over 100 birds, on the effect of various levels of subcutaneously administered reserpine on actual thyroxine secretion rates and also on the rate of release of thyroxine from the thyroid gland. The feeding was well controlled and the experimental set-up was generally of the factorial design. ^{131}I was used as a tracer in the determination of the rate of release of organically bound ^{131}I and the thyroxine secretion rate. A goitrogen (Tapazole or Thiouracil) was administered in part of the trials to enhance the sensitivity of thyroidal-^{131}I determinations. Reserpine at levels of 30 μg/100 g/day and above depressed the thyroidal-^{131}I release rate in chickens and caused body wt. losses due to inanition. No level of reserpine was found (range employed 5–100 μg/100 g body wt.) which would inhibit thyroid function without at the same time depressing feed intake, suggesting therefore that thyroid inhibition at higher levels of reserpine administration is only secondary to inanition.

1. PIPES *et al.* (1957), *Poultry Sci.*, **36**, 1149.

352 Observations on the Control of Aldosterone Secretion in the Dog. M. HOLZBAUER and M. VOGT (United Kingdom).

Experiments on the mechanisms involved in the increase of aldosterone secretion after severe haemorrhage have been carried out. They were done on anaesthetized dogs and aldosterone was estimated in adrenal venous blood by a chemical method. It was found that splanchnotomy abolishes the rise in aldosterone secretion; the reason for this is being investigated. Observations on the distribution of aldosterone between cells and plasma of adrenal venous blood have shown that large and variable amounts of aldosterone are present in the cells and that estimations of aldosterone in plasma alone can lead to erroneous conclusions.

353 The Effects of Vasopressin on the Adrenals and Testes of Lesioned and Hypophysectomized Rats. S. LEVINE, H. GOLDMAN and M. ALPERT (United Kingdom).

Although there has been considerable controversy concerning the role of vasopressin in the regulation of secretion of ACTH, the available evidence indicates that vasopressin may play a role in regulation of ACTH secretion. The present experiments investigated whether vasopressin can affect the surgically blocked elaboration of ACTH.

Male albino rats were assigned to the following groups: (1) lesioned; (2) lesion, vasopressin; (3) hypophysectomized; (4) hypophysectomized,

vasopressin; and (5) normal. The lesions destroyed the infundibular stem and resulted in marked diabetes insipidus, adrenal and testicular atrophy. Two weeks following either lesion or hypophysectomy all animals were unilaterally adrenalectomized. Half the lesioned and hypox animals were treated daily with pitressin sufficient to reduce the urine output and water consumption to near normal. The remaining animals received no treatment. After seven days the second adrenal was removed along with the testis. Both adrenals were weighed and then examined histologically.

The results indicate that the non-pitressin treated lesioned and hypox animals showed no compensatory adrenal hypertrophy. The same occurred in hypox pitressin treated animals. The pitressin treated lesioned animals did show significant compensatory adrenal hypertrophy. Histological examination of their adrenals indicated the second adrenal indistinguishable from normal, whereas the first adrenal of these subjects and the first and second adrenals of all the remaining animals were markedly atrophied and abnormal histologically.

These data tend to indicate that vasopressin plays some role in ACTH regulation although the evidence does not warrant the conclusion that vasopressin is *the* neurohumour responsible for this control. Further, the results contradict recent conclusions that vasopressin acts directly on the adrenal.

354 Spinal Neurones: Synaptic Connexions in Relation to Chemical Transmitters and Pharmacological Responses. J. C. Eccles (Australia).

Whenever they have been investigated adequately, all spinal neurones receive a diversity of synaptic connexions. This will be illustrated with reference to motoneurones, to the cells of origin of ascending tracts such as the ipsilateral cutaneous and the dorsal and ventral spino-cerebellar tracts, to interneurones and to Renshaw cells. With all these types of cell there is evidence that more than one type of synaptic transmitter acts directly on the cell. Besides the two different classes of transmitters concerned in excitatory and inhibitory synaptic action respectively, there is also evidence that different excitatory transmitters act on some cells. For example, it seems that not all excitatory synapses on Renshaw cells are cholinergic. There is also convincing evidence that different types of primary afferent fibres may make direct synaptic connexion on many types of nerve cell, e.g. the cells of origin of dorsal and ventral spino-cerebellar tracts and some interneurones, but as yet there is only suggestive evidence that the different species of primary afferent fibres operate by different excitatory transmitter substances.

There are two types of synapses on spinal neurones that have clear pharmacological properties. It is well established that motor axon collaterals excite Renshaw cells cholinergically. The evidence for this rests on comprehensive pharmacological studies: the actions of acetylcholine and related substances; the action of cholinergic blocking substances; the action of anti-cholinesterases. Similar studies have failed to detect cholinoceptive synapses on either spinal motoneurones or interneurones. All inhibitory synapses exhibit specific pharmacological responses that suggest the action of the same unique transmitter: inhibitory synaptic action is depressed both by strychnine, brucein and related substances and by tetanus toxin. Besides these specific pharmacological actions related to synaptic transmitter substances, there are general pharmacological actions on nerve cells that apparently are related not to receptor sites for synaptic transmitter substances, but to receptor sites dispersed over the cell surface, probably on non-synaptic areas. This is well established for the depressant action of GABA and related substances, but it also seems to be the case with the excitatory action of the acidic amino-acids, though it is still conceivable that the excitatory transmitter substance or substances in the spinal cord may be related to the acidic amino-acids.

355 Discussion of the Previous Paper. The Pharmacology of the Centrifugal Acoustic System in the Cat's Brain Stem. J. P. Desmedt (Belgium).

356 Neuropharmacological Analysis of Strychnine-like Drugs. V. G. Longo (Italy).

Recent investigations have shown the particular features of the convulsant action of strychnine; these appear to be related with a blockade of inhibitory systems. This has been demonstrated both at spinal[1] and supraspinal levels.[2]

In a series of neuropharmacological investigations carried out in our laboratory, the convulsant properties of strychnine have been demonstrated in other natural and synthetic compounds.[3, 4, 5, 6] The characterization of strychnine-like activity has been based on the following tests: (1) occurrence of hyperreflexia and tonic seizures during toxicity trials in laboratory animals; (2) appearance in the electroencephalogram of a sustained paroxysm of 30 c/s waves at spinal, cerebellar and mesencephalic levels; and (3) increase of the polysynaptic spinal reflex and blockade of the spinal "primary" inhibition.

As a result of these studies, this characteristic activity has been recognized for four different types of chemical structures, viz: strychnine, thebaine, 5-7-diphenyl-1-3-diazadamantane-6-ol (1757 IS), 4-phenyl-4-formyl-N-methyl piperidine (1762 IS).

The ability to reproduce the pattern of strychnine intoxication with synthetic substances of a relatively simple chemical structure should be of help for the investigations of the structural and biochemical

features of the inhibitory mechanisms at the level of the central nervous system, as well as for identifying the actual inhibitory transmitter.

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2. Desmedt, J. E. and Monaco, P. (1960), *Arch. Int. Pharmacodyn.*, **129,** 244.
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6. Longo, V. G. and Pinto Corrado, A. (1961), *Proc. Soc. Exp. Biol. Med.* In press.

357 The Influence of Some Pharmacological Agents on the Cholinoreactive and Adrenoreactive System of the Reticular Formation and Some Other Parts of the Brain. P. P. Denisenko (U.S.S.R.).

Experiments on rabbits and cats, as well as on such preparations as "encéphale isolé" and "cerveau isolé" have shown that cholinomimetics and adrenomimetics, such as acetylcholine, arecholine, nivalin, adrenaline, phenamine, and some others produced, in a marked degree, desynchronization of the reticular-formation biopotentials and the cerebral cortex.

Administration of central cholinolytics or adrenolytics induces synchronization with a predominance of slow high amplitude potentials in EEG. Activation of the cerebral cortex by stimulation of the cervical sympathetic nerve can be prevented and abolished not only by means of adrenolytics (aminazine), but central cholinolytics (methyldiazil, benactyzine, etc.) as well. Central cholinolytics and adrenolytics exert a blocking action upon the effect elicited in the midbrain reticular formation after direct electric stimulation. While adrenolytic blockade lasts, phenamine and adrenaline fail to produce any stimulating effect, on the other hand, anticholinesterases, as well as acethylcholine and arecholine, elicit a definite arousal response. Adrenaline and phenamine effect is in no way impaired by blocking the cholinoreactive systems. This antagonism is most apparent in experiments on the "encéphale isolé" and the "cerveau isolé".

The data obtained, together with facts from the literature, enable the author to infer that cholinoreactive as well as adrenoreactive systems of the reticular and other formations of the cerebrum play a definite role in the arousal response in the cortex. It may be supposed that transmission of nervous impulses from one section of the cerebrum to another is effected mainly through cholinergic channels, the adrenoreactive systems being engaged mainly in trophic adaptation processes.

358a Uber die quantitative Nachweisbarkeit der positiv inotropen Wirkungen von Herzglykosiden am Herzlungenpraparat von Meerschweinchen bei verschiedenen Zufuhrungsarten. H. Iwata und H. Klupp (Germany).

Meerschweinchen wurden in Urethannarkose künstlich beatmet und nach Klupp und Kieser ein Herzlungenpräparat hergestellt. Arterieller Widerstand und venöser Einflussdruck blieben bei allen Versuchen konstant. Gemessen wurde das Minutenvolumen. Die Herzlungenpräparate wurden nach Beendigung der Präparation durch langsame Infusion einer 1 prozentigen Lösen von Bromallylbutyl-barbitursäure (0·05 cml/min) langsam vergiftet. Der Beginn der Insuffizienz des Herzens ist an der Abnahme des Minuten-volumens erkennbar. In Kontrollversuchen wurde gefunden, dass die tödliche Dosis der Barbitursäure in einer mathematisch erfassbaren Beziehung zu der Dosis steht, welche das Minuten-volumen auf die Hälfte einschränkt. Wenn zu diesem Zeitpunkt ein wirksames Glykosid dem venösen Vorratsgefäß zugesetzt wird, erhöht sich die Lebensdauer des Präparates und damit die zum Tod notwendige Dosis der Barbitursäure. Als Überlebensdauer wird die Differenz zwischen tödlicher Barbituratdosis bei den Kontrollversuchen und den Versuchen mit den einzelnen Dosen der verwendeten Glykoside bezeichnet. Sie steht innherhalb eines bestimmten Dosenbereichs in linearer Beziehung zu den Logarithmen der verabreichten Glykosiddosen. Da der Glykosidzusatz bei allen Präparaten zu einem Zeitpunkt erfolgt, in dem derselbe Insuffizienzgrad erreicht ist, kann die Wirkung der gleichen Dosen an verschiedenen Präparaten gut verglichen werden. Die relative Wirkungsstärke einzelner Glykoside kann statistisch mit einer Varianzanalyse errechnet werden.

Es wird über die Verwendbarkeit der Methodik zum Nachweis der peroralen, intramuskulären und intraperitonealen Resorption der Herzglykoside bei Vorbehandlung der Tiere zu verschiedenen Zeiten berichtet.

358b The Quantitative Assessment of the Positive Inotropic Action of Heart-Glycosides on the Guinea-Pig Heart-Lung Preparation with Various Methods of Administration. H. Iwata and H. Klupp (Germany).

Guinea pigs were given artificial respiration under urethane narcosis, and a heart-lung preparation was made by the Klupp and Kieser method. Arterial resistance and venous pressure remained constant in all experiments. The cardiac output was measured. The heart-lung preparation was subsequently slowly poisoned by means of a slow infusion of a 1 per cent solution of bromallyl-butyl-barbituric acid (0·05 ml/min). The beginning of cardiac insufficiency could be recognized by the decrease of the minute-volume. It was found in

control experiments that the lethal dose of barbituric acid is mathematically proportional to the dose which halves the cardiac output. If an effective glycoside is added to the venous storage vessel at this time, the life-span of the preparation increases and with it the dose of barbituric acid required to cause death. The difference between the lethal barbiturate dose in control experiments and in experiments with various doses of the glycosides used was designated the survival time. Within a certain range of dosages there is a linear relationship between them and the logarithms of the glycosides administered. As the addition of glycosides in all preparations was carried out at the time when the same degree of insufficiency had been reached, the effect of equal doses on different preparations can easily be compared. The relative effectiveness of individual glycosides can be statistically calculated by analysis of variance.

An account is given of the peroral, intramuscular and intraperitoneal resorption of heart-glycosides as preliminary treatment of the animals at various periods.

359 A Comparison of Some Properties of g-Strophantin and Convallatoxin on the Heart Lung Preparation of the Cat. K. Pávek, V. Dittertová and F. V. Selecký (Czechoslovakia).

Heart-lung preparations of cats were damaged by means of phenobarbital sodium and the effects of g-strophanthin and convallatoxin on the damaged hearts was compared.

The decreased minute volume and cardiac output was restored by both drugs, but convallatoxin acted much more rapidly and its negative chronotrophic potency was more marked. The same occurred with the blood pressure, the lowered pressure due to the barbiturate being restored to normal by both drugs but again convallatoxin acted the more rapidly. The increased venous pressure was decreased by both drugs in a like manner.

For therapeutic use, lower doses of convallatoxin than of g-strophanthin are necessary but even so disturbances of cardiac action and cardiac arrest are produced by lower doses of convallatoxin than g-strophanthin.

The comparative value of both glycosides will be discussed.

360 Digitoxin-(23-^{14}C). G. Rabitzsch (Germany).

The preparation of ^{14}C-labelled cardenolide glycosides was previously done either by the biological method (digitoxin-U-^{14}C)[1] or by partial synthesis of the monoglucoside from inactive aglycone and D-glucose-U-^{14}C.[2] The labelling of the biologically less important part of the molecule makes this compound less suitable for the examination of pharmacological questions. Therefore, the possibility of labelling a native glycoside specifically with ^{14}C in the aglycone moiety was investigated, using digitoxin (digitoxigenin-(3-D)-tridigitoxoside). After blocking the digitoxose hydroxyl groups the labelling of the steroid skeleton of the intact glycoside is done by ozonolytic degradation of the lactone ring to the ketolacetate,[2] followed by resynthesis to the unsaturated lactone with ethyl bromoacetate-^{14}C according to Reformatzky. The separation of the heterogeneous reaction product is performed by thin-layer and column chromatography with silica gel. Studies on the binding of this compound and its aglycone to heart muscle are in progress in this laboratory.

1. Okita *et al.* (1954).
2. Turba and Scholtissek (1954).

361 The Relationship between Molecular Structure and Pharmacological Activity in Derivatives of Digitoxigenin and Digoxigenin. A. Stafford and S. E. Wright (Australia).

Most of the present knowledge about structural requirements for cardiac glycoside-like activity is derived from measurement of lethal doses in the cat. This work was done to see whether similar structure activity relations hold for the positive inotropic effects.

Oxidation or acetylation of the 3-OH of digitoxigenin or the 3- and 12-OH of digoxigenin caused a reduction in toxicity in the cat, guinea pig and isolated embryonic chick heart to 1/10–1/12 of that of the parent aglycone without altering qualitatively the electrocardiographic changes. The concentrations required to produce positive inotropic effects on the isolated cat papillary muscle and guinea-pig heart were 2–20 times higher than for the parent compounds but even at their most effective concentrations they produced a significantly smaller increase in the force of contraction.

Removal of the 14-OH and formation of a *trans* C-D ring junction resulted in compounds with no demonstrable toxicity in the cat or guinea-pig, 1/20–1/50 of the activity of digitoxigenin or digoxigenin on the embryo chick heart, and only a negative inotropic action on the isolated guinea-pig heart and cat papillary muscle.

Hydrogenation of the lactone ring reduced toxicity both in the cat and the guinea-pig and reduced the positive inotropic activity to 1/50–1/100 of the parent compound.

These results suggest that positive inotropic activity in the cardiac glycosides parallels their toxicity.

362 The Therapeutic-Toxic Ratio in Digitalization. P. Szekely and N. A. Wynne (United Kingdom).

The therapeutic and toxic effects of digitalis have been studied in man and in the experimental animal

over the past ten years, with special reference to the state of the myocardium, electrolyte shifts and body temperature.

A number of patients with advanced myocardial disease and atrial fibrillation who developed ventricular rhythms after conventional maintenance doses, have subsequently been maintained in a good state of compensation with satisfactory control of the heart rate on very small doses of digitalis which did not produce toxic arrhythmias. Ca potentiated the therapeutic action of digitalis and it also increased its toxic effects. Hypocalcaemia reversed the digitalis effect on the electrocardiogram and eliminated digitalis-induced cardiac arrhythmias. K seemed to have no inhibitory influence on the therapeutic action of digitalis, but it eliminated cardiac arrhythmias caused by digitalis. K deficiency proved to be an important factor in the causation of digitalis arrhythmias. Mg eliminated digitalis-induced cardiac arrhythmias. Lowering of the body temperature decreased the susceptibility to digitalis toxicity.

These findings are in agreement with the fundamental studies of Lown *et al.*[1] and Rothlin *et al.*[2] It seems that digitalis toxicity is but an extension of its therapeutic effects and that cardiac and primary extracardiac factors play an important role in determining whether a given digitalis dose is therapeutically inadequate, therapeutically active or toxic.

1. Lown, B. and Levine, S. A. (1955), *Current Concepts in digitalis therapy*. Churchill, London.
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363a Accroissement de la Toxicite de la Digitaline chez le Cobaye par l'Iproniazide et la Phenylisopropylhydrazine. J. Jacob et P. Echinard-Garin (France).

Chez le cobaye, l'iproniazide (phosphate: 200 mg/kg s.c.) et la phenyisoprophlhydrazine (sulfate: 20 et 40 mg/kg s.c.) ont augmenté, de façon modérée mais statistiquement significative, la toxicité de la digitaline Nativelle administrée par infusion intraveineuse lente.

Dans le cas de l'iproniazide, l'effet a été plus important 16 et 24 heures que 1 et 2 heures après l'injection de l'inhibiteur des monoamine-oxydases. Dans celui de la phenylisopropylhydrazine, il était par contre très net dès après 1 ou 2 heures. Dans les deux cas il persistait au moins pendant 4 jours.

Ces caractéristiques chronologiques correspondent à celles qui sont connues pour l'inhibition *in vivo* des mono-amine-oxydases per ces agents. Il est donc possible que le phénomène observé soit en relation avec l'augmentation des amines catéchiques cardiaques, mais d'autres interprétations doivent aussi être considérées, en particulier des modifications dans la distribution ou le métabolisme de la digitaline.

363b Increased Toxicity of Digitaline in the Guinea Pig due to Iproniazide and Phenylisopropylhydrazine. J. Jacob and P. Echinard-Garin (France).

In the guinea-pig, iproniazid (phosphate: 200 mg/kg s.c.) and phenylisopropylhydrazine (sulphate: 20 and 40 mg/kg s.c.) caused a moderate but statistically valid increase in the toxicity of the digitaline, Nativelle, administered by slow intravenous infusion.

In case of iproniazid the effect was more marked 16 and 24 hr after injection of the inhibitor of mono-amine-oxydase than 1 to 2 hr after it. On the other hand, in the case of phenylisopropylhydrazine it was very marked after 1 or 2 hr. In both cases it persisted for at least 4 days.

These chronological features correspond to those which are known to occur with *in vivo* inhibition of mono-amine-oxydase by these agents. Thus it is possible that the phenomenon which was observed is related to an increase of the cardiac catecholamines, but other interpretations must also be considered, particularly modifications in the distribution or metabolism of digitalines.

364a Die Wirkung des Digitoxins auf die Acetylcholinsynthese in vitro. F. Švec (Tschecho-Slovakei).

Von verschiedenen Seiten wurde bereits früher nachgewiesen, dass eine Komponente der komplexen Digitaliswirkung einen Synergismus mit der Acetylcholinwirkung bzw. eine Sensibilisierung der Erfolgsorgane für das Acetylcholin darstellt. Es blieb die Frage offen ob die Digitalisglykoside die biologische Synthese des Acetylcholins beeinflussen oder das Freisetzen von aktiven Acetylcholin von den gebundenen Acetylcholin beschleunigen.

Es wurde die Beeinflussung der Acetylcholinsynthese im künstlichen Enzymsystem durch Digitoxin *in vitro* geprüft. Die purifizierte Cholinacetylase wurde aus dem Meerschweinchengehirn, das Koenzym A aus der Leber gewonnen. Es hatte sich gezeigt, dass das Digitoxin die Acetylcholinsynthese in verschiedenen Dosen nicht beeinflusst. In natürlichen Homogenaten von Gehirnsubstanz mit intakten Nervenzellen übt das Digitoxin eine zweifasige Wirkung aus. In kleinen Dosen (1–10 μg/g) bewirkt das Digitoxin eine Beschleunigung, in grossen toxischen Dosen (50–100 μg/g) eine Hemmung der Acetylcholinsynthese.

In einem Enzymsystem in welchem die Cholinacetylase aus einer durch Azeton getrockneten Gehirnsubstanz, das Koenzym A aus frischer Leber gewonnen wurde, ist Digitoxin wirkungslos. Wenn zu diesem Medium ein frisch zubereitetes Homogenat aus Herzmuskel, welches Homogenat intakt Zelle bzw. subzelluläre Organellen enthält zugefügt wurde, erhöhte sich die Synthese des Acetylcholins durch Digitoxin.

Es konnte angenommen werden, dass das Digitoxin den Acetylcholinstoffwechsel im Sinne

einer Beschleunigung der Acetylcholinsynthese nur bei Vorhandensein von Zellen bzw. zellulären Organellen beeinflusst.

364b In vitro Effect of Digitoxin on Acetylcholine Synthesis. F. Švec (Czechoslovakia).

It has already been demonstrated in various quarters that a component of the complex action of digitalis is synergism with the action of acetylcholine or sensitization of the effector organs to acetylcholine. It is debatable whether the glycosides of digitalis influence the biological synthesis of acetylcholine or accelerate release of active acetylcholine from the bound form.

The influence exerted *in vitro* by digitoxin on acetylcholine synthesis in an artificial enzyme system has been examined. The purified cholinacetylase was obtained from the guinea-pig brain, coenzyme A from the liver. It was demonstrated that various doses of digitoxin did not affect the synthesis of acetycholine. In natural homogenates of brain matter with intact nerve cells digitoxin had a biphasic action. In low doses (1–10 μg/g) digitoxin brought about acceleration, in large toxic doses (50–100 μg/g) inhibition of acetylcholine synthesis.

Digitoxin was ineffective in an enzyme system in which the cholinacetylase was obtained from acetone-dried brain matter and coenzyme A from fresh liver. When to this medium freshly prepared homogenate of heart muscle containing intact cells or subcellular organella was added, synthesis of acetylcholine was enhanced by digitoxin.

It can be assumed that digitoxin affects acetylcholine metabolism in acceleration of acetylcholine synthesis only in the presence of cells or cell organella.

365 Comparative Studies on the Pharmacological Activity of a Pure A-Series of Digitalis Glycosides. Y. Aramaki and K. Kikuchi (Japan).

According to our serial study on cardiac glycosides, digitoxigenin has almost the same potency as digitoxin when administered intermittently at a shorter interval, 2·5 min or less, than that of the pigeon method designed in USP XIV and the activity of digitoxin is only slightly superior to that of digitoxigenin when cat papillary muscle is used *in vitro*. The exceedingly low activity of digitoxigenin is seemingly due to its minimal accumulation and/or the rapidity of its inactivation.

An attempt to evaluate the activity of pure samples of an A-series of digitalis glycosides containing digitoxigenin as aglycone was made using our new method.

Materials: (1) Purpurea glycoside a (purity 98·8 per cent), (2) Lanatoside A and α-acetyl digitoxin (pure crystal), (3) Digitoxin (purity 99 per cent).

Methods: LD of the glycosides in pigeons were determined with various injection intervals either shorter or longer than 5 min. Accumulation rate was measured by determining the additive LD at 3–72 hr after a single dose of 50 per cent pigeon unit. Sum total LD was figured out by daily successive small doses.

Results: Contrary to the widely-held view, purpurea glycoside A and lanatoside A displayed rather higher activities than digitoxin and the accumulation of the former two was considerably lower than that of the latter suggesting that the lower activity of the former two in clinical use is due to their low accumulation. On the other hand, α-acetyldigitoxin was slightly inferior to digitoxin in potency but their accumulation was comparable to each other.

366 Helveticoside, a Cardiac Glycoside from Erisymum canescens Roth. A. Babulová, L. Buran and F. V. Selecký (Czechoslovakia).

Helveticoside was isolated from *Erysimum canescens* Roth, which grows in Slovakia. Biological assays with helveticoside of other origins have been performed only in cats.

Therefore, a comparative study of cardiotoxic activity of helveticoside was performed in pigeons, guinea pigs, cats and monkeys. As helveticoside is supposed to have a g-strophantin-like activity, the cardiotoxicity was compared with g-strophantin and convallatoxine.

In pigeons, the most toxic was convallatoxine. The toxicity of g-strophantin and helveticoside was equal, but less than that of convallatoxine. In guinea pigs, the most potent was again the convallatoxine, followed by g-strophantin, whilst the toxicity of helveticoside was considerably less. In cats the most toxic was again convallatoxine, followed by helveticoside, and the least toxic was g-strophantin. In monkeys the most toxic was convallatoxine, followed by g-strophantin and the least toxic was helveticoside.

Electrocardiographic changes associated with helveticoglycoside were as typical as with the other cardiotonics.

The experiments led us to two conclusions: (a) helveticoside isolated from our plant has true cardiotoxic properties; and (b) when new cardiotoxic drugs are investigated for their cardiotoxic potency it is necessary to use several species.

367 Some Properties of the Cardio-active Substance in Spleen. L. B. Cobbin and R. H. Thorp (Australia).

A cardio-active substance has been demonstrated in acetone extracts of freeze-dried ox spleen,[1] which differs from the usual pharmacologically active substances found in that organ,[2] and from the catecholamines.[3]

The cardio-activity of the crude extract resembles that of the cardiac glycosides, for the extract causes positive inotropic effects on isolated papillary muscles and perfused mammalian hearts. Suitable

doses increase the stroke volume of the kitten heart-lung preparation and increase the amplitude of cardiac contractions in anaesthetized guinea pigs.

The active principle is soluble in acetone, water and methanol, but appears to be only sparingly soluble in chloroform. It is ultrafilterable through a cellophane membrane. The cardio-activity is not destroyed by heating for one hour at 100°C in acid solution, but becomes progressively destroyed as the pH of the solution is raised.

The substance differs from the usual polypeptides which stimulate plain muscle for it resists destruction by the proteolytic enzymes trypsin and chymotrypsin, even after 48 hr exposure. Additional evidence that it is not a polypeptide has been obtained from a paper chromatographic system.

It has been demonstrated that the active substance has a basic nature as a result of its behaviour on paper electrophoretograms and ion-exchange resin. It can be partially purified from contaminating material on a silica gel column, and eluted with mixtures of methanol and chloroform. By combining ion-exchange resins and chromatographic columns, considerable purification may be achieved.

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2. Cobbin, L. B. and Thorp, R. H. (1959), *Brit. J. Pharmacol.*, **14,** 392.
3. Cobbin, L. B. and Thorp, R. H. (1960), *Nature (Lond.)*, **186,** 473.

368 A Comparative Study of Two New Glycosides Peruvoside and Ruvoside from Thevetia neriifolia, Juss. N. N. De, M. M. Vohra and J. D. Kohli (India).

Pharmacological action of two new glycosides, peruvoside and ruvoside,[1] has been studied. The chemical constitution of peruvoside and its relationship with other previously known thevatia glucosides has been worked out by Bloch *et al.*[2] According to Rangaswami and Rao[3] ruvoside is the corresponding alcohol of peruvoside.

Evidence of cardiotonic activity was obtained by direct and indirect techniques such as the isolated papillary muscle of the cat, isolated hypodynamic guinea-pig heart, ECG changes in the cat, etc. Biological activity was assayed in cats, guinea pigs and pigeons. Both the glycosides were found to be nearly as potent as ouabain. By differential techniques and back titration in animals, the oral absorption, duration of action and cumulative toxicity of the two glycosides were measured. A comparison between the two glycosides and the clinically-important glycosides digoxin and ouabain has been carried out. Data obtained suggest that peruvoside is a good, quick acting glycoside which is well absorbed orally and has low cumulative toxicity. Ruvoside, however, appears to possess no advantage over the other quick-acting glycosides.

1. Rangaswami and Rao (1958), *J. Sci. Industr. Res.*, **17B,** 331.
2. Bloch *et al.* (1960), *Helv. Chim. Acta.*, **43,** 652.
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369a Considérations sur l'Activité Biologique des Nouveaux Glucosides Cardiotoniques de la Digitale Pourpre: Gitaloxine et Vérodoxine. A. Georges, J. Page et G. Duvernay (Belgium).

L'activité biologique des extraits de glucosides secondaires de la digitale pourpre n'est pas liée directement au seul pourcentage des glucosides totaux, vu l'activité biologique fort différente et la proportion variable de chacun des glucosides constitutifs de ces extraits.

Cette activité diffère aussi sensiblement d'après le choix de l'animal test car il existe, pour un même glucoside cardioactif, de grosses variations de susceptibilité entre animaux d'espèces différentes.

Ainsi, chez le cobaye, la gitaloxine et la vérodoxine sont respectivement 1,2 et 5 fois plus actives que la digitoxine tandis que chez la souris la gitaloxine est 8 fois plus active et la vérodoxine approximativement aussi active que la digitoxine.

L'activité biologique de tels extraits de glucosides cardiotoniques n'est pas—tout au moins chez le cobaye—due exclusivement à l'addition des activités propres de chacun des glucosides qui les composent mais également à une synergie évidente entre glucosides.

Cette synergie est nulle entre la digitoxine, la gitoxine, la gitaloxine et le strospéside, mais apparait immédiatement en la présence de vérodoxine qui confère à chacun de ces glucosides un important surcroit d'activité.

La raison de cette synergie n'est pas encore connue. Elle dépend à la fois du rapport entre la quantité de vérodoxine et des autres glucosides et du degré d'activité spécifique des glucosides présents dans le mélange.

369b Some Aspects of the Biological Activity of New Cardiac Glycosides of Purple Digitalis: Gitaloxine and Verodoxine. A. Georges, J. Page and G. Duvernay (Belgium).

The biological activity of glycosidic extracts from digitalis is not directly related to the percentage of total glucosides, as the proportion of the glucosides of which the extracts are composed are variable. Their activity also varies noticeably with different animals used for testing, as there is a wide variation in the susceptibility of animals of different species to the same glucoside.

Thus, gitaloxine and verodoxine are 1, 2 and 5 times more active in the guinea pig than digitoxine,

while in the mouse gitaloxine is 8 times more active and verodoxine and digitoxine have approximately the same degree of activity.

The biological activity of such cardiotonic glucoside extracts is not entirely due to an additive effect of each of the glucosides contained by them but also to an obvious synergism between glucosides (this applies to guinea-pigs at least).

This synergism is nil between digitoxiline, gitoxine, gitaloxine and stropeside, but it appears as soon as verodoxine is present, as the latter causes a marked increase in the activity of each of the other glucosides.

The reason for this synergism is not yet known. It depends both on the ratio of verodoxine to other glucosides and on the degree of specific activity of the glucosides present in the mixture.

370 Demethylation and Methylation of Drugs and Physiologically Active Compounds. J. Axelrod (U.S.A.).

Most drugs (analgesics, narcotics, antihistamines, sympathomimetic amines) having an *N*- or *O*-methyl group undergo demethylation in the organism. Depending on the structure of the administered drug, the resulting metabolic product is less active, equally active or more active than the parent compound. A family of enzymes that can carry out the demethylation reaction to yield formaldehyde, and the corresponding phenols or nor-derivatives will be discussed. These enzymes are localized in the microsomes of the liver and require both reduced triphosphopyridine nucleotide as well as oxygen.

Many physiologically active compounds (adrenaline, noradrenaline, histamine, *N*-acetylserotonin) are methylated on the phenolic or nitrogen group in the body. These *O*- and *N*-methylated metabolic products can be active or less active than the parent compound. A group of enzymes called methyltransferases carry out their methylation reaction by transferring the methyl group of *S*-adenosylmethionine to an oxygen or nitrogen group. Catechol-*O*-methyl transferase *O*-methylates catechols, requires divalent metals, and is widely distributed; hydroxy-indole-*O*-methyl transferase *O*-methylates *N*-acetylserotonin to form the hormone melatonin and is found only in the pineal gland; imidazole-*N*-methyltransferase *N*-methylates histamine and is widely distributed; phenylethanolamine *N*-methyltransferase *N*-methylates norepinephrine as well as other phenylethanolamine derivatives and is highly localized in the adrenal medulla.

371 Discussion of the Previous Paper. Z. M. Bacq (Belgium).

372 Desulphuration. R. D. O'Brien (U.S.A.).

Desulphuration of the phosphorothionate insecticides (e.g. parathion malathion) is an activation step, i.e. it converts these materials from extremely weak to very potent anticholinesterases, and so determines their toxicity. The desulphurating system of mammals is located in liver microsomes, and requires oxygen, reduced pyridine nucleotide, magnesium and nicotinamide. Desulphuration rates depend upon age, sex and species. They can be modified by sex hormones, methylcholanthrene, SKF 525A and ethyl *p*-nitrophenyl phenylphosphorothionate (EPN). Studies *in vivo* will be reported, but their interpretation is made difficult by the inevitable concurrence of degrading action both on the parent material and its desulphuration product. The role of desulphurating systems in determining variations of toxicity among species will be discussed.

373 The Enzymatic Conversion of Cyanide to Thiocyanate. B. Sörbo (Sweden).

The conversion of cyanide to thiocyanate represents an important detoxification mechanism for cyanide in higher animals. Two different enzymes can accomplish this conversion. One, named rhodanese, is found mainly in liver and kidney. This enzyme has been isolated and crystallized, and the properties of crystalline rhodanese will be reviewed. The enzyme can use thiosulphate, thiosulphonates or inorganic polysulphide as sulphur donor, but not colloidal sulphur (in contrast to earlier reports in the literature). Sulphite can replace cyanide as sulphur acceptor. Spectrographic analysis has demonstrated the absence of metals from the crystalline enzyme and no evidence for the presence of a prosthetic group rhodanese has been obtained. Inhibition studies suggest that the active group is a disulphide bond. Possible reaction mechanisms for the enzyme will be discussed.

The other enzyme, mercaptopyruva-cyanide *trans*-sulphurase, forms thiocyanate and pyruvate from cyanide and β-mercaptopyruvate. This enzyme occurs in liver and kidney but in contrast to rhodanese, significant amounts of activity are also found in the erythrocytes. The enzyme has not been isolated, but purification and inhibition studies suggest that it contains copper and that sulphydryl groups are necessary for the activity. This enzyme may be identical with other *trans*-sulphurases, which transfer the sulphur from mercaptopyruvate to sulphite or mercaptans.

The biological function of the thiocyanate-forming enzymes, their role in cyanide poisoning and their therapeutic applications will be discussed.

374 Discussion of the Previous Paper. T. P. Lapin (U.S.S.R.).

375 Esterase Action. W. Kalow (Canada).

Recent studies with electrophoretic methods have made it clear that different tissues of any given

animal species contain a number of esterases which have not yet been properly characterized. With some notable exceptions, the best known are the esterases which occur in plasma and erythrocytes. Corresponding plasma esterases of different species are usually similar but perhaps never identical. This accounts for the species differences in the metabolic fate of ester-type drugs; e.g. procaine is hydrolysed in man but appears intact in the urine of horses; atropine is metabolically converted but not hydrolysed in man while it is rapidly destroyed by atropin-esterase in some rabbits. Furthermore, hereditary differences of esterases between subjects of a given species cause some individual variation in the response to pharmacologically active esters. For some series of drugs, the duration of action within a given species has been correlated with *in vitro* hydrolysis rates. Such observations have been recorded, e.g. for some organic phosphorous insecticides, some local anaesthetic esters, and for muscle relaxants of the type of succinylcholine. However, even when esterase action obviously influences the duration of the drug response, the esterase does not necessarily terminate the drug action, e.g. the pseudocholinesterase in man quickly reduces the plasma concentration of injected succinylcholine but the pharmacological effect of the drug seems to be terminated by diffusion away from the receptor organ.

376 Discussion of the Previous Paper. K. B. Augustinsson (Sweden).

377 Enzymes that Inactivate Active Polypeptides. E. G. Erdös (U.S.A.).

Three features of the interplay between proteolytic enzymes and polypeptides are currently of especial interest: (1) Proteases—and also esterases—release biologically active peptides; (2) Trypsin, chymotrypsin, carboxypeptidase, aminopeptidase and other endo- and exo-peptidases are used in elucidating polypeptide structures; and (3) In the circulation and in tissues enzymes metabolize active peptides and thus may affect the duration of action. Many enzymes exhibit more than one of these features, but the third feature will be discussed in detail. There are also numerous enzymes, e.g. leucine aminopeptidase and plasmin, which are frequently determined in normal and clinical conditions. A relation with activity towards polypeptides is of interest here.

The substrates which have been reviewed include a variety of peptides of different activity with mol. wt. up to that of corticotropin. Polypeptides of known structure such as angiotensin, bradykinin, corticotropin, oxytocin, vasopressin, etc., and some less well-known ones of probable future importance, e.g. "Kinins", Substance P, have been surveyed. The enzymes which metabolize these agents are often named after one substrate, for example: angiotensinase, kininase, oxytocinase, or vasopressinase. Consideration is given to recent notable progress made in purifying oxytocinase and in establishing its bond specificity as well as to the characterization of the human plasma enzyme, which destroys the activity of bradykinin.

378 Data on the Effects of Some Pharmacological Agents on Cholesterol Metabolism and Blood Lecithin. N. A. Kharauzov (U.S.S.R.).

Investigation of the influence exerted by pharmacological agents on cholestrol metabolism and on blood lecithin are of primary importance in the search for successful means capable of affecting the onset and development of atherosclerosis, since impaired cholesterol metabolism has been shown to be the main factor in the causation and development of atherosclerosis. The literature also contains ample evidence on the importance of the relationship between blood cholesterol and lecithin for the development of atherosclerosis, its progress being inhibited by a relative rise of the lecithin level as compared with that of cholesetrol.

The effect of 6 pharmacological agents on blood lecithin have been investigated under the experimental conditions known as N. N. Anichkov's model of cholesterol atherosclerosis in rabbits. Lecithin levels were estimated in rabbits in terms of blood phosphorus, assayed by Fiske and Subbarow's method.

Among the agents under investigation, dibazole has displayed a considerable blood cholesterol raising effect following its repeated administration to rabbits. This resulted in a proportionate decrease in the cholesterol: lecithin index, followed by appreciable inhibition in the development of experimental atherosclerosis.

Data reported in the literature indicated the possibility of cholesterol synthesis being inhibited by phenyl-ethyl acetic acid and by vanadium. According to our data, inhibition of cholesterol synthesis may be achieved by the administration of vanadium, while phenyl-ethyl acetic acid administration results in a certain degree of cholesterol break-down as well as in inhibition of its synthesis. An especially high degree of cholesterol break-down has been shown to follow the simultaneous administration of phenyl-ethyl acid and vanadium sulphate.

Available evidence warrants the expectation that on lowering the dosage of phenyl-ethyl acetic acid, its toxic effects should be reduced so that its administration may be combined with that of vanadium, providing an effective means for bringing the development of atherosclerosis under control.

379 Influence of Androsterone on Blood Cholesterol, Phospholipids, and Lipoproteins of Cholesterol-fed Cockerels. H. Y. C. Wong, T. F. Johnson and F. Johnson (U.S.A.).

It has been reported by Cook *et al.*[1] as well as from our laboratory[2, 3] that administration of

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testosterone reduced the blood lipids and severity of atherosclerosis of cholesterol-fed chickens. Furthermore, Cook reported[4] that androsterone had no effect on the plasma lipids of cholesterol-fed cockerels. However, androsterone inhibited coronary but not aortic atherosclerosis of cholesterol-fed cockerels. The present study was undertaken to determine the influence of androsterone on blood cholesterol, phospholipids, and lipoproteins whether on plain mash or fed an "atherogenic" diet of 2% cholesterol and 5% cottonseed oil added to the mash for 34 weeks. Four groups of 8 cockerels to a group were used in the experiment: (I) Controls on plain mash; (II) Plain mash plus 1·25 mg of androsterone; (III) Cholesterol-fed only; and (IV) Cholesterol-fed plus 1·25 mg of androsterone. At the end of 8 weeks, the following results were obtained (1) There were no significant differences in th body wt., blood cholesterol, phospholipids, and lipoproteins of any of the groups studied whether on plain mash or an "atherogenic" diet treated with androsterone or not; and (2) androsterone did not reduce the severity of aortic atherosclerosis. Histological sections of aorta and coronary arteries will be presented and discussed.

(Supported by grants-in-aid from National Heart Institute, H-2420 (C4), Kerr Chickeries, Washington Heart Association, and Wesson Oil Co.)

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380 Effect of β- and ω-Substituted Long Chain Fatty Acids upon Experimentally Induced Hypercholesterolaemia. K. H. Harper and W. A. Broom (United Kingdom).

The effect of unsaturated long chain fatty acids in reducing the blood cholesterol level in animals with induced hypercholesterolaemia is now well known. As yet, however, no one has succeeded in establishing the *modus operandi* although various speculations have been made. The evidence would now appear to be overwhelmingly in favour of a participation of the unsaturated portion or portions of the molecule in the cholesterol lowering process although this is disputed by several workers.

It was our premise, suggested by Rimington, that if normal metabolic destruction of the fatty acid molecule could be prevented, then a more sustained and possibly more active compound would be obtained. The major route in the metabolism of such compounds is β- and ω-oxidation and we have therefore tested several derivatives of unsaturated long chain fatty acids, in which such a process might be expected to be hindered if not totally prevented.

The method adopted for testing these chemicals upon hypercholesterolaemia was a modified form of that described by Hauge[1] using rats as the experimental animal. A negative and positive control group (gum acacia and cod liver oil respectively) have been included in each experiment and our findings on any particular compound are related to these two standards. Other compounds, including an inhibitor of cholesterol synthesis, and certain proprietary products have also been tested and results with these are also reported and discussed.

1. Hauge and Nicolaysen (1959), *Acta physiol. scand.*, **45,** 26.

381 Natural and Synthetic Isoprenoids, and Lipid Metabolism. E. Adami and F. Piccinini (Italy).

A series of products, structurally related to natural poly-isoprenic compounds, was synthesized. *In vitro* and *in vivo* studies were carried out on the influence of such substances and of other natural isoprenoids upon the metabolism of cholesterol and fatty acids and upon the ubiquinone and tocopherols contents of several organs.

382 Comparative Hypocholestereolaemic, Oxygen Consumption and Anti-Goitre Activities of Thyroxine Analogues in the Rat. E. C. Jorgensen, C. Greenberg, N. Zenker and P. Lehman (U.S.A.).

A series of aliphatic, alicyclic and aromatic ethers of 3,5-diiodotyrosine has been synthesized and tested for effects on plasma cholesterol, oxgen consumption and thiouracil-induced goitre in the rat. The aliphatic and alicyclic analogues were without thyroxine-like activity at levels of some 200 times an effective dose of thyroxine.

Aromatic ethers showed activity ranging from antagonistic to thyromimetic. The most potent of the thyromimetics were 3,5-diiodo-2′,3′-dimethyl-L-thyronine and 3,5-diiodo-4(4′-hydroxy-1′-naphthoxy)-DL-phenylalanine.

In no case has any compound been demonstrated to exhibit only one thyromimetic parameter of activity throughout its entire dose range. However, some analogues are capable of displaying a single biological activity at certain doses. This may provide an eventual, absolute separation.

Within the present series, structural requirements associated with the phenolic ring of thyroxine and responsible for the various hormonal effects tested, are very similar. These include: (a) aromatic character; (b) 4′-hydroxylation, or this position capable of metabolic hydroxylation; (c) for maximal activity, a substituent in the meta position of the phenolic ring capable of positioning distal in space to the alanine-bearing ring.

383a Die Underschiedliche Wirkung von Thyroxin-Homologen auf den Blutcholesteringehalt und den Sauerstoffverbrauch. A. Bänder (Deutschland).

Bei der Untersuchung einer grossen Zahl von thyroxinverwandten Verbindungen wurde beobachtet, dass die Senkung des Blutcholesterinspiegels nicht immer mit einer Steigerung im Sauerstoffverbrauch parallel geht. So fanden wir Substanzen, die den Sauerstoffverbrauch der behandelten Tiere unverändert liessen und dabei den Cholesterinspiegel um mehr als 20 Prozent senkten. Bei weiteren Untersuchungen, vor allem bei chron. Verabreichung wiesen diese Verbindungen eine erhebliche Toxizität auf. So wurden unter anderem histo-pathologische Veränderungen in der Leber, Nebennierenrinde und den männlichen Keindrüsen gesehen.

383b Variable Effect of Thyroxin Homologues on Blood Cholesterol and Oxygen Consumption. A. Bänder (Germany).

Investigation of a large number of compounds related to thyroxin it was shown that the fall in the blood cholesterol level does not always occur in parallel with an increase in oxygen consumption. We were able to find substances which left unchanged the oxygen consumption of treated animals but reduced the cholesterol level by more than 20 per cent. In further investigations, in particular with chronic administration, these compounds displayed considerable toxicity: among other things histopathogical changes in the liver, suprarenal cortex and male testicular glands.

384 The Effects of 3:5:3′—Triiodo-D-thyronine (D-T_3) in Thyroid Deficiency. J. M. Merrill and J. Lemley-stone (U.S.A.).

In patients with myxoedema, 5 μg/kilo per day of D-T_3 increases oxygen consumption. This finding raised the possibility that in thyroid deficiency the hypolipaemic and calorigenic effect of D-T_3 may not be independent. To investigate this relationship, hypothyroidism was induced in rats by giving 900 μ c ^{131}I intraperitoneally. After ten weeks, hypothyroidism was apparent from growth curves and total body oxygen consumption. The oxygen consumption of the control rats was 0·777 ± 0·07; the thyroid deficient rats, 0·482 ± 0·04 ml/g/hr.

The effect of D-T_3 on: (1) total body oxygen consumption; (2) Qo_2 of myocardial homogenates; (3) incorporation of ^{14}C labelled acetate into liver cholesterol and fatty acids; (4) total cholesterol and fatty acids of the liver and serum, was studied in half of the thyroid deficient animals. Absence of functioning thyroid tissue was confirmed microscopically after sacrifice.

Administration of the D-T_3 was associated with an increase in total body oxygen consumption, increased Qo_2 of myocardial homogenates, increased incorporation of ^{14}C into liver cholesterol and fatty acids with no statistically significant change in cholesterol or fatty acids of the liver.

These results suggest that in thyroid deficient states the lipid and calorigenic effects of D-T_3 are closely related.

385 Effect of Thyroid on Serum Cholesterol and Bile Acids. K. Hellström, S. Lindstedt and J. Sjövall (Sweden).

Patients with hypo- and hyper-function of the thyroid gland have been studied with respect to serum cholesterol levels and bile acid composition. The ratio of glycine to taurine conjugated bile acids in 10 hypothyroid patients was 8 : 8 before and 3 : 4 after treatment (in normal cases previously studied the ratio was 3 : 1). Oral administration of taurine resulted in a ten-fold decrease in the ratio but did not seem to influence serum cholesterol levels. The ratios between cholic, chenodeoxycholic and deoxycholic acids were 1·1 : 1·0 : 0·9 before and 0·9 : 1·0 : 0·7 after treatment.

After feeding ^{14}C-labelled cholic acid the half-life and production of bile acids were determined. The half-life was 4·1 days (normal 2·8) and the daily production was 0·37 g (normal 0·71 g). After treatment the values tended towards the normal range. After treatment of hypothyroidism with D-triiodothyronine the half-life of cholic acid became longer than before the treatment.

386 Some Observations on the Relationships between Atherosclerosis, Hyperlipaemia and D-Thyroxine. O. F. Davis, C. Beck, N. Sloan, M. Bergal and A. J. Levine (U.S.A.).

The constellation of factors reportedly influential in atherogenesis is considerable and varied. Important relationships have been established between atherosclerosis and age, sex, diet, psychopathology, ethnic background, weight, body type, other somatic pathology (e.g. endocrinopathies such as hypothyroidism) and specific serum lipids.

The factors which encourage the propensity to the earliest proliferative changes in artery (atherogenesis) should be differentiated from those processes which enhance an ongoing atherosclerosis or promote recurrent infarcation. There is a large body of evidence pointing to hyperlipaemia as conducive to heightened arteriosclerosis.

This communication describes long-term studies in animals and 239 humans with a new lipaemia-clearing compound. An average drop of 105 mg/% in serum cholesterol supervened within 35 days.

With strict reservations as to the aetiological importance of hyperlipaemia per se; but, with an acute awareness of the role played by these lipids in

furthering an extant atherosclerosis, we are encouraged to recommend the compound studied for further investigation.

387 Nuclear Magnetic Resonance Studies of the Binding Sites of Biologically Active Substances. I. Method and Protein Binding. O. JARDETZKY, J. J. FISCHER and P. PAPPAS (U.S.A.).

When the thermal motion of a chemical group is restricted, the relaxation time of the nuclear spins which it contains is shortened and the nuclear magnetic resonance (NMR) peak attributable to that group is correspondingly broadened. While in the case of compounds such as water, the NMR spectrum of which consists of a single peak, the interpretation in terms of specific binding is complicated by non-specific effects, such as change of viscosity or diamagnetic anisotropy of the solute, in the case of small molecules (mol. wt. up to 600) yielding spectra which contain more than one peak, it is possible to correct for these effects by taking the relative broadening of the peak compared to that of another from the same molecule. In the case of nonspecific effects, the ratio of line widths (at half height) for any two peaks from the molecule remains unchanged by changes in the composition of the solution. Selective broadening of one or more peaks can thus be interpreted in terms of specific interactions involving the chemical groups to which the peaks correspond. The stoichiometry of the binding can be established by plotting relative line width (or its inverse, the relaxation time) against the relative concentration of the interacting molecular species, the plot showing an inflexion point at the value of the relaxation time corresponding to the stoichiometric ratio of the complex formed. The method has been used to investigate the protein binding of several penicillins, streptomycin and the tetracyclines. Proton magnetic resonance spectra of the antibiotics in aqueous solutions were obtained under conditions of varying protein (egg or serum albumin) concentration, pH and ionic strength and the groups attached to the protein determined.

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388 Nuclear Magnetic Resonance Studies of the Binding Sites of Biologically Active Substances. II. The Epinephrine-Adenosinetriphosphate System. N. WEINER, P. PAPPAS and O. JARDETZKY (U.S.A.).

Epinephrine and adenosine triphosphate (ATP) are present in heavy granules of the adrenal medulla in high concentrations, and an ionic interaction between these two substances has been postulated as a mechanism of epinephrine storage in this gland.[1, 2] The chemical nature of this interaction can be investigated by nuclear magnetic resonance (NMR). The NMR spectrum of solutions of epinephrine in heavy water consists of four peaks, the first of which is attributed to the protons attached to the aromatic ring; and the others to the protons attached to the alpha, beta and methyl carbons of the side chain. These peaks are identified according to the chemical shifts and spin-spin coupling constants. With increasing concentrations of ATP, a selective broadening of the triplet beta carbon peak and doublet alpha carbon peak is observed. The pattern of broadening is consistent with a two-point stabilization of the epinephrine molecule across the hydroxyl and amino groups of the side chain. The interaction thus appears to involve more than a single group on the epinephrine molecule, e.g. a simple ionic bond, since the selective broadening would then be confined to the peak due to the immediately adjacent group. On the other hand, there is no broadening of the peak attributed to the ring spectrum, which indicates the absence of a chemical interaction involving the hydroxyl groups of the catechol moiety.

(Supported by National Science Foundation Research Grant 9563 and USAF SAM Contract AF-41 (657)-197.)

1. BLASCHKO *et al.* (1957), *J. Physiol.*, **139,** 316.
2. CARLSSON *et al.* (1957), *J. Biol. Chem.*, **227,** 243.

389 Sterical Structure and Activity of Catecholamines on α- and β-Receptors. E. J. ARIËNS (Holland).

The ratios for the activity of the stereo isomers of catecholamines differ for the effects induced on α- and β-receptors. The L/D ratios are: arterenol 4, epinephrine 8 for the contraction of the vas deferens of the rat (α-receptors) and arterenol 50, epinephrine 50, isuprel 500 for the increase in frequency and contraction of the guinea-pig auricle (β-receptors). The activity of dopamine (desoxy-arterenol) is on both receptors practically equal to that of D-arterenol. The *N*-aralkyl substituted arterenols, e.g. *p*-oxyphenyl*iso*propylarterenol (Cc 25) behave as sympatholytics on the α-receptors. The intrinsic activity is lost, the affinity maintained. The L/D ratio for Cc 25 in this respect is 1:10. This means an inversion in the ratio: a switch of the highest activity in the L-series for the α-sympathomimetic arterenols to the D-series for the α-sympatholytic derivatives. Isuprel behaves as a compound with an intermediate intrinsic activity. This is found to be the result of it being a mixture of an L-compound which acts like arterenol, as an α-sympathomimetic, and a D-compound, which acts like Cc 25, as an α-sympatholytic. In the series of L-*N*-aralkylarterenols the intrinsic activity is still present in the isopropyl derivative, in the D-series it is lost already in the *iso*propyl compound. This preindicates the inversion in the L/D ratio mentioned. The β-sympathomimetic action is maintained throughout the series of *N*-substituted compounds. DL-Cc 25 is even more active than DL-isuprel in this respect.

Here dichloro-arterenol acts as a specific antagonist, a β-sympatholytic.

The study of the β-mimetic action of the optical isomers of the *N*-alkyl substituted arterenols leads to interesting conclusions.[1] The relations described may give information on the receptors for the catecholamines and the interaction between drug and receptor.

Table 1

	α-mimetic	α-lytic	β-mimetic	β-lytic
arterenol L/D	4		50	
epinephrine L/D	8		40	
isuprel L/D	L-mimetic	D-lytic	500	
C_c 25 L/D		1/10	1000	
DL-dichloro-arterenol				lytic

1. Claassen, V. Personal communication.

390 Stereo-Specific Divergence in the Actions on Adrenergic α- and β-Receptors. V. Claassen (Holland).

The natural occurring catechols arterenol and epinephrine both show an α-mimetic and β-mimetic activity. In these cases both activities are located in the same optical antipode. Until now no racemic compounds were known that showed an interaction with both α- and β-receptors and in which these interactions require a different sterical configuration.

In *p*-oxyphenyl*iso*propylarterenol (Cc 25), a N-aralkyl substituted arterenol derivative, there is found an α-sympatholytic activity and a β-sympathomimetic action.

Moed *et al.* prepared L-Cc 25 and D-Cc 25 by introduction of a (racemic) *p*-hydroxyphenyl-*iso*propyl group into L-arterenol and D-arterenol, respectively. In this case the α-lytic activity appeared to be located in the diastereoisomere mixture sterically related to D-arterenol (Ariëns) while the β-mimetic action was dominantly present in the diastereoisomers related to L-arterenol.

This stereo-specific divergence in the effects induced on α- and β-receptors by the antipodes of a pure racemic compound was found separating the optical antipodes of 2-(phenoxy-2-propyl-amino)-1-(*p*-hydroxyphenyl)-1-propanol HCl (Duvadilan), also prepared by Moed. In the compound sterically related to L-arterenol the β-mimetic activity was present while this activity was almost lacking in the antipode. The α-lytic activity appeared to be situated in the compound related to D-arterenol.

Table 1

Activity ratio	α-lytic activity	β-mimetic activity
L Duvadilan/D Duvadilan	0·02	40
L-*Cc* 25/D-*Cc* 25	0·10	30

These relations between sterical structure and the actions on α- and β-receptors opens new possibilities for studying the chemical properties of the receptor and the relationship between drug and receptor.

391 New Factors in Structure-Activity Relationships of Adrenergic Blocking Agents. R. P. Ahlquist and B. Levy (U.S.A.).

Structure–activity studies of the adrenergic blocking agents have until recently been confined to blockade of responses associated with only one type of adrenergic receptor—in our terminology, the α-receptor. The introduction of the dichloro derivative of isoproterenol (DCI) has opened the new field of β-adrenergic receptor blockade. We have found at least two compounds that block both α- and β-receptors; namely, methoxyphenamine (Orthoxine) and dihydroergotamine. To determine the total adrenergic blocking activity of any compound we have developed a method using four different adrenergic responses and four different adrenergic activators. In dogs, anaesthetized with either morphine–pentobarbital or morphine–chloralose, by suitable electronic transducers we have simultaneously recorded arterial pressure, heart rate, intestinal activity, and the response of the retractor penis. The standard adrenergic activators were epinephrine, isoproterenol, phenylephrine and ethylnorepinephrine. Using criteria that we have previously published we can determine the type of adrenergic blockade produced, the relative potency, and the duration of action. We have also been able to exclude as adrenergic blocking agents certain compounds that others have considered to be β-adrenergic blocking agents.

(The study was supported by Grant H-3514 from the National Heart Institute, U.S. Public Health Service.)

392 On the Mechanism of Development of Adrenaline Action at the Site of "Adrenaline-Receptor" and on the Relation of the Action to the Activity of O-Methyltransferase. R. Imaizumi (Japan).

The following experimental results have led to a hypothesis that Fe^{++}-protein is a constituent of so-called adrenaline receptor:

(1) Adrenaline action is weakened by the addition of α,α′-dipyridyl and orthophenanthroline to the perfusate, but is strengthened by the slight addition of Fe^{++} in Pissemski's test.

(2) Adrenaline combines with Fe^{++} in the mol ratio of 2:1 making chelate compound.

(3) Protein prepared from heart muscle combines evidently with adrenaline in the presence of Fe^{++}.

Furthermore, the relation between O-methyltransferase and its related several substances was investigated.

Following are the facts clarified by *in vitro* experiments using adrenaline as the substrate of O-methyltransferase and by Pissemski's test:

(1) The methylation of adrenaline is inhibited by pyrogallol and such catechol derivatives as catechol, protocatechu aldehyde, protocatechuic acid, gentisic acid, etc.

(2) The action of adrenaline is weakened by the addition of such divalent cations as Mn^{++}, Mg^{++}, Co^{++}, Ni^{++}, Fe^{++}, etc.

(3) The co-factors above mentioned do not manifest any influence upon the action of synephrine and tyramine.

(4) The vasoconstrictive action of adrenaline, tyramine, synephrine and $BaCl_2$ is potentiated by the addition of Cr^{+++} and hydroquinone, but Cr^{+++} and hydroquinone indicate no relation with O-methyltransferase activity.

393 Interaction of Adrenaline with Amino-acids and Receptors in the Blood Stream. B. N. Manukhin (U.S.S.R.).

The interaction of adrenaline with 14 amino-acids was studied. Except for tyrosine and leucine, all the amino-acids studied were found under experimental conditions to bind adrenaline to complexes with the dissociation constant (K_d) from $4{\cdot}10 \times 10^{-3}$ M to $1{\cdot}31 \times 10^{-5}$ M. The dissociation constant of adrenaline–amino-acid complexes in the case of alanine (K_d $2{\cdot}04 \times 10^{-3}$ M) and its derivatives is shown to depend on functional amino-acid groups. K_d drop to $1{\cdot}3 \times 10^{-5}$ M takes place when incorporating a sulphhydryl group (cysteine into the alanine molecule. Other functional groups give intermediate K_d values, with the exception of the hydroxyphenyl group which in the β-position completely eliminates the ability of tyrosine to bind adrenaline. Calculations show that one amino-acid molecule binds in this position with one adrenaline molecule.

In experiments on spinal rabbits the dependence of the value of the pressure response upon the adrenaline amount was followed up. One adrenaline molecule is shown to bind with one molecule of hypothetic adrenoreceptor to a complex with K_d of $3{\cdot}2 \times 10^{-7}$ M, the adrenaline amount being optimal.

The interaction of adrenaline with the receptor is regarded, according to Koshtoyant's hypothesis on the transmission of nervous action, as a first step in the participation of this mediator in biochemical processes of the effector.

394 Chemical Models of Drug-Receptor Interaction. A. Gero (U.S.A.).

In a paper,[1] a model study of drug–receptor interaction is reported in which amino-acids (representing the receptor) were allowed to react with diethylaminoethanol (representing the drug) in a two-phase system consisting of water and CCl_4. The equilibrium distribution of the drug model was always displaced in the direction from CCl_4 to water; amino-acids containing OH, amide, or peptide groups were especially effective in causing such displacement. The results were interpreted to demonstrate ionic attraction and repulsion, and hydrogen bonding, between the amino-acids and the drug model.

Similar experiments with dibutylaminoethanol as the drug model yielded quite different results. Only amino-acids with sizeable alkyl groups, such as leucine and isoleucine, were capable of drawing this drug model into the aqueous phase, and only at relatively low pH. All other amino-acids, even those with strongly hydrogen-bonding groups, were ineffective. The obvious meaning of these findings is that dibutylaminoethanol, being more lipophilic than diethylaminoethanol, resists transfer into the aqueous phase unless the molecules of the receptor model also have sufficient lipophilic character; even then, interaction between the drug model and the receptor model occurs only at relatively low pH where the drug model is considerably ionized and therefore relatively hydrophilic.

Applied to interaction between actual drugs and receptors, this again emphasizes the role of ionic charges on the drug and also the importance of the hydrophilic or lipophilic character, not only of the drug, but also of the receptor and its vicinity.

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395 A new concept of the Interrelationships between Vasodilator Receptors. R. A. Woodbury and C. B. Nash (U.S.A.).

In dogs where adrenergic blockade had been established with phentolamine, neostigmine Br 0·08 mg/kg, pilocarpine HBr 0·5 mg/kg, or methacholine 0·04 mg/kg practically eliminated the depressor activity of epinephrine. Atropine sulphate 2 mg/kg re-established the depressor activity of epinephrine.[1] Though it is possible that these cholinergic drugs block the β-receptors of the sympathetic nervous system and that atropine removes this block, the following hypothesis which explains these data is offered. A receptor mechanism (name suggested is MU), is shared by the β-receptors and the cholinergic receptors. This MU-receptor serves in the final common path involved in the relaxation of blood vessels. Activation of the cholinergic receptors by pilocarpine and other similar drugs gains dominant control of the MU-receptors so that the β-receptors are unable to influence the MU-receptors and, therefore, effective vasodilation action by the β-receptors is prevented.

Studies of the coronary flow in dogs show that in the presence of excessive cholinergic activity,

epinephrine produces a relative decrease in coronary flow in contrast with the effect of epinephrine under control conditions. A possible relationship of these observations to angina pectoris will be discussed.

(Acknowledgment is given for support from the American Heart, Tennessee Heart and Memphis Heart Association.)

1. Woodbury (1959), *Fed. Proc.*, **18,** 461.

396 Pharmacological Differentiation of Phenethylamines Commonly Conceived to Have the Same Modes of Action. B. B. Clark, P. M. Lish and K. W. Dungan (U.S.A.).

The isolated seminal vesicle of the rat, which consists of smooth muscle apparently devoid of β receptors and which is not relaxed by adrenergic inhibitory agents, was employed to characterize the smooth muscle relaxant action of a phenethylamine vasodilator isoxsuprine.[1, 2] It is difficult to distinguish this type of drug from isoproterenol when studied on smooth muscle densely populated with β receptors. The dichloro-analogue of isoproterenol blocks both the effects of isoxsuprine and isoproterenol on tracheal smooth muscle.[3] The smooth muscle of the seminal vesicle can discriminate between the adrenergic agents phenylephrine and methoxamine commonly thought to have the same mode of action.

Tests in this laboratory on the seminal vesicle *in situ* have defined to a significant extent, and for the first time, the responses of this organ to a variety of hormones and drugs injected into the arterial blood supply. This tissue does not respond to histamine or the ganglionic stimulants DMPP and nicotine. It is highly sensitive and contracts sharply to L-epinephrine, L-norepinephrine, acetylcholine, direct electrical depolarization, or electrical stimulation of the sympathetic nerve.

Studies with sympatholytic, adrenergic, and cholinergic blocking agents suggest that an adrenergic mediator is involved even when contractions are induced by acetylcholine or direct depolarization. Discrimination can be made between drugs having different proportions of adrenergic and papaverine-like smooth muscle relaxant activities and between relatively pure adrenergic blocking agents and those having significant smooth muscle depressant action.

1. Lish (1960), *J. Pharmacol.*, **129,** 191.
2. Ariens (1960), *Arch. int. Pharmacodyn.*, **127,** 479.
3. Dungan (1961), *J. Allergy*, **32,** 139.

398 Influence of Phenol and Alcohol on the Action of Various Intestinal Stimulants Analysed by the Concentration-action Curve. H. Matsumoto and S. Suganuma (Japan).

To evaluate the view that the presence of an N atom in a drug molecule is important for its affinity to certain receptors, the N-free compounds (phenol and alcohol) were examined for their influence on the concentration–action (C–A) curves of intestinal stimulants. The stimulants used were ACh, pilocarpine, $BaCl_2$ and nicotine with rabbits and histamine, in addition, with guinea pigs. Results:

(1) The C–A curves of the above stimulants fitted in with the formula $Kx^n = y/y^{max} - y$ (x: concentration, y: intensity of action) with or without phenol or alcohol.

(2) The idea that the sensitivity of the tissue to drugs should be distinguished into two factors, "susceptibility" and "reactivity", was supported especially by the shift patterns of the C–A curve indicating the co-existence of synergism and antagonism obtained in some of the interactions of drugs examined.

(3) The antagonistic factor suggesting a competitive mechanism was detected in all of the drug interactions tested with guinea pigs and in some with rabbits. Hence, the presence of an N atom seems to be not indispensable, at least for a drug molecule to exhibit the affinity to the receptors of the stimulants used.

(4) A striking difference has been obtained between ACh and pilocarpine as regards the modification of their C–A curves produced by phenol with rabbits. This implies that there may exist a special drug interaction, which is incompatible with the usual anatomical concept of the site of action of drugs.

399 Reactivation of Alkylphosphorylated Cholinesterase by a Constituent of Liver. L. V. Neuhoff and H. Kewitz (Germany).

The dialysate of liver homogenate contains a substance which reactivates alkylphosphorylated cholinesterase. The reactivation has been measured by use of a preparation of partially purified acetylcholinesterase from pigs' brain inhibited by paraoxon.

From the dialysate, 20 fractions have been separated by high voltage electrophoresis at pH 4–5. The reactivating factor is among the six anionic compounds. None of the cationic constituents have been found to reactivate. A similar separation of the acid substances from the basic compounds can be performed by use of a column of aluminium oxide pretreated with acetic acid. The basic compounds are not bound to the column. The anionic substances, however, can be eluated from the column with diluted ammonia. The reactivating capacity is entirely stable against hydrolysis for 12–15 hr at 120°C in 6 N HCl. Hydrolysis of the cationic fraction liberates a reactivating compound which cannot be distinguished from the anionic reactivator. It has been concluded that liver tissue contains the reactivating substance in the free and in the bound form. The free reactivator has the properties of an organic acid which cannot be hydrolysed neither by strong alkali nor by strong acids and which is stable to heat. In the bound form the reactivator has basic properties.

400 Metabolism of ^{14}C-labelled N-methylpyridinium-2-aldoxime. I. Enander, A. Sundwall and B. Sörbo (Sweden).

N-methylpyridinium-2-aldoxime iodide (PAM) labelled with ^{14}C on the methyl group was injected into rats and the excretion of ^{14}C was followed in the urine. Paper chromatography of the latter demonstrated four peaks. The major one corresponded to unchanged PAM and one had an Rf-value identical with that of *N*-methylpyridinium-2-nitrile. The identity of the latter was also established by chemical reactions. A third peak corresponded to *N*-methylpyridinium-2-carboxylic acid (homarine) and a fourth peak had an Rf-value identical with *N*-methyl-α-picolinium-amine. Reversed isotope dilution demonstrated, however, that very small amounts of this compound were formed. Reversed isotope dilution also demonstrated the formation of small amounts of *N*-methylpyridinium-2-pyridone.

401 1:1′ - Trimethylene bis(4 - formylpyridinium bromide) dioxime in Anticholinesterase Poisoning. M. P. Milošević and V. Vojvodić (Yugoslavia).

The brain and blood cholinesterase activity of mice poisoned with about equilethal doses ($2LD_{50}$) of five organophosphorus anticholinesterase compounds was measured electrometrically immediately after death. In animals poisoned with armine, phosphamidone, dimefox and dipterex, cholinesterase activity in the brain was reduced to 28, 20, 25 and 19 per cent of normal, while OMPA reduced cholinesterase activity to 57 per cent of normal values. Enzyme activity in the blood was in a instances reduced to under 30 per cent of normal activity.

Simultaneous treatment of animals with 25–50 mg/kg of 1:1′-trimethylene bis(4-formylpyridinium bromide)dioxime (TMB-4) without atropine, offered complete protection in animals poisoned with armine, phosphamidone and dipterex. No satisfactory protection was obtained against dimefox and OMPA. In armine poisoned animals, treated with TMB-4, there was definite reactivation of phosphorylated cholinesterase in the blood, but not in the brain. However, in mice poisoned with phosphamidone or with dipterex, in spite of the marked reduction in mortality which occurred with TMB-4, there was no reduction in cholinesterase inhibition either in the brain, or in the blood.

Complete protection with TMB-4 was obtained also against $2LD_{50}$ of carbaminoylcholine, but not against acetylcholine. In animals poisoned with some nonphosphorus anticholinesterase compounds (neostigmine, piridostigmine, demecarium, isolan, dimethan) TMB-4 offered definite protection only in neostigmin intoxication.

402 Relation between the Chemical Structure and the Acute Toxicity in the Series of Alkyl-Alkoxy Derivatives of p-Nitrophenyl-phosphoric acid and the Protective Effects of PAM-2, TMB-4 and Atropine. V. Vojvodić, D. Likar, Z. Binenfeld and M. Stevanović (Yugoslavia).

LD_{50} values were determined in white mice by different routes of administration. The following compounds were investigated: ethyl-ethoxy-*p*-nitrophenyl phosphoric acid (I); methyl-ethoxy-*p*-nitrophenyl phosphoric acid (II); methyl-*iso*propoxy-*p*-nitrophenyl phosphoric acid (III); and diethoxy-*p*-nitrophenyl phosphoric acid (IV) as the standard compounds. All compounds studied were more toxic by s.c. than by i.p. application. The I_{50} values, like the LD_{50} values, demonstrated a definite relationship with the chemical structure of the compounds. Atropine, PAM-2 and TMB-4 show a highly protective effect in poisoning with compounds I–IV. The best results were obtained when the oximes were given 30 min before the poisons. No protective effect was observed when oximes were administered 1 hr before.

The optimal doses of PAM-2 and TMB-4 were about a fifth of their LD_{50}. TMB-4 showed a markedly greater protective effect than PAM-2.

403 Effects of Oximes and Atropine on the Toxicity of Tertiary and Quaternary Organophosphorous Cholinesterase Inhibitors which yield the Same Phosphorylated Cholinesterase. A. Sundwall (Sweden).

The therapeutic effect of N-methylpyridinium-2-aldoxime methanesulphonate (P2S) and atropine in mice and rabbits poisoned with methyl-*iso*propoxyphosphorylfluoride (sarin), *iso*propoxy-2-dimethylaminoeththiomethyl-phosphine oxide (37 S-N) and methyl-*iso*propoxyphosphoryl thiocholine (37 S-N^+) has been studied. (These three inhibitors yield methyl-*iso*propoxy phosphorylated acetocholinesterase). P2S alone was 5–10 times as effective against the quaternary compound (37 S-N^+) as against the tertiary (37 S-N). Addition of atropine to the treatment enhanced the LD_{50} of the tertiary compounds considerably but had much less effect on the LD_{50} of the quaternary compound.

In anaesthetized cats poisoned with lethal doses of 37 S-N^+ plasma concentrations of about 2×10^{-5} M (4 μg/ml) of P2S were needed to counteract bradycardia and respiratory failure.

The neuromuscular block produced by the three inhibitors on the rat phrenic nerve diaphragm preparation was reversed by P2S in concentration 5×10^{-6} to 1×10^{-5} M. No difference in the time required to produce 50 per cent reversal could be demonstrated for the different inhibitors if equimolar concentrations of the latter were used.

404 Oxime-Atropine Therapy of Anticholinesterase Poisoning. J. F. O'Leary, A. M. Kunkel, A. H. Jones and L. M. Somers (U.S.A.).

Fifty per cent of rabbits treated intravenously

with a mixture of pyridine-2-aldoxime methochloride (2-PAM), 1:1-trimethylenebis (4-formylpyridinium chloride) dioxime (TMB4), and atropine 30 sec following intravenous poisoning with *iso*propylmethylphosphonofluoridate (Sarin) survived doses of greater than 200 times the LD_{50} of the latter without resort to artificial respiration. Under such conditions, complete survival of animal groups was obtained with the mixed oximes after 60 LD_{50}'s of Sarin, but after only 18 LD_{50}'s with 2-PAM alone and 8 LD_{50}'s with TMB4 alone. The mixed oximes, in contrast to the single ones, saved a large percentage of dogs receiving 70 LD_{50}'s of ethyldimethylphosphoramidocyanidate (Tabun). The ability of the 2-PAM type of oxime to potentiate sympathetic and adrenergic responses in animals, either alone or in a mixture with TMB4, will be discussed in relation to its possible role in the superior anticholinesterase antagonism by the mixed oximes.

405 Effect of Some Drugs on the Amount of Cerebral Acetylcholine in Rats. Z. Fink, V. Vondráček, J. Tulach and M. Sajda (Czechoslovakia).

Influence of a number of drugs with anticholinergic effect on the amount of cerebral acetylcholine was studied. According to the anticholinergic effect, one group of the drugs under examination can be classified as parasympathicolytics structurally related to acetylcholine. A second group of drugs belongs to the so-called cholinesterase reactivators; it is represented by some oxims. In the above-mentioned drugs, the relationship to cholinesterase was evaluated *in vitro* and *in vivo* and an attempt was made to prove their influence on acetylcholine biosynthesis.

406 The Antagonism of "Irreversible Anticholinesterase" Agents by Certain Quaternary Oximes. E. Bay (U.S.A.).

Rapid reactivation of cholinesterase after its inhibition by long-acting anticholinesterase agents is not only of theoretical interest but also has clinical value in several conditions: (1) "Cholinergic crisis", (2) Accidental exposure to organophosphorous insecticides, and (3) "Nerve gas" poisoning. Systematic search has resulted in the recognition of two oximes as particularly effective adjuncts to atropine in the management of the above-mentioned circumstances, 2-formyl-1-methylpyridinium chloride oxime (2-PAMCl) and 1:1' trimethylenebis (4-formylpyridinium bromide) dioxime (TMB-4). These oximes are weak anticholinesterase compounds and also receptor blockers. TMB-4 has greater specificity for ganglia than 2-PAMCl and mimics the action of curare at the neuromuscular junction. On the other hand, 2-PAMCl has a weak ganglionic blocking effect and a "dual" action at the motor end-plate. If the undesirable hypotensive action of TMB-4 is counteracted with simultaneously administered Vasoxyl (2,5-dimethoxynorephedrine) or if the competitive blocking component of the dual action of 2-PAMCl at the neuromuscular junction is supplemented by administering such compounds as pentamethonium, the oximes' life saving capacity in atropinized rabbits poisoned with *iso*propyl methylphosphorous fluoridate (Sarin) is increased. The neuromuscular blockade during the early stages of poisoning by organophosphorous anticholinesterases is antagonized by both the reactivating and the acetylcholine-blocking properties of the oximes. Our results indicate that the beneficial action of the oximes on long-established neuromuscular block by anticholinesterase compounds is due only to their abilities to reactivate cholinesterase.

407 The mode of Action of Diacetylmonoxime (DAM) as an Antidote against Sarin Poisoning. E. M. Cohen, P. J. Christen and E. Mobach (Holland).

DAM has been shown to protect cholinesterase in the sarin poisoned rat rather than to reactivate the phosphorylated enzyme. Although in earlier experiments[1] inactivation of sarin by a direct chemical interaction with DAM could not be excluded, this cannot be the only mode of action of DAM as in other species DAM is much less effective than in the rat.

Myers[2] found that DAM is able to reactivate sarin inhibited plasma tributyrinase in the rat and, to a minor degree, in the mouse. The mode of action of DAM suggested by this author consists of "catalysing" the hydrolysis of sarin by dephosphorylation of the phosphorylated and continuously rephosphorylated tributyrinase in the blood stream.

In order to evaluate the importance of a plasma component as a factor in the mechanism of action of DAM, we compared the speed of hydrolysis of ^{32}P labelled sarin in the presence of DAM in plasma from different species with that in water. Separation of sarin from hydroxysarin was achieved by freezing and drying the incubation mixtures at about 0·01 mm Hg and −180°C. The radioactivity of the non-volatile hydroxysarin was measured after deproteinization of the mixtures.

The results in the main support the view of Myers: in rat plasma hydrolysis of sarin was complete within one min, that in plasma from the other species amounted to 50–80 per cent at exactly one min after the addition of DAM and in water, hydrolysis proceeded at a much lower rate. Irreversible inhibition of tributyrinase by triorthocresylphosphate only partly abolished the effect of DAM, however.

In addition, the effects of inhibition of phosphofluoridase and pseudocholinesterase on our system will be discussed.

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2. Myers, D. K. (1959), *Biochem. Biophys. Acta*, **34**, 555.

408 Beta-Phenethylbiguanide Degradation Products and Their Biological Activity. A. N. Wick, J. L. Faymon and C. J. Stewart (U.S.A.).

Beta-phenethylbiguanide produces hypoglycaemia in diabetic animals but also inhibits glucose oxidation *in vitro*. In an attempt to reconcile the *in vivo* and *in vitro* actions, studies have been made with the drug labelled in the α-position with ^{14}C. The radioactive biguanide was administered both orally and intraperitoneally to Sprague–Dawley rats at a dosage of 100 mg/kl. Following oral administration, the animals were sacrificed at zero time, 1/2, and at 2 hr. The radioactive components in the gastric juice were purified via paper chromatography and the ultra-violet (U.V.) and infrared (I.R.) spectra taken. The I.R. spectra indicated the presence of the aromatic group at zero time, a decreased aromatic absorption at 1/2 hr, and an absence of the aromatic group at 2 hr. Two substances, Fraction I and Fraction II, were isolated from 24 hr urine samples after oral administration. The I.R. spectra revealed the presence of the aromatic and guanidine groups in Fraction I, and the presence of the guanide group but the absence of a conjugated system and the aromatic group in Fraction II. Intraperitoneally injected animals were sacrificed after 1/2 hr. I.R. spectra of the purified radioactive spot (IPGJ), indicated the absence of the aromatic group. The U.V. band at 233 mμ demonstrated the presence of the conjugated guanidine group. The effect of IPGJ, on the rate of oxygen uptake by Ehrlich ascites tumour cells was investigated. IPGJ increased oxygen uptake by 52 per cent whereas an equal weight of β-phenethylbiguanide inhibited it by 71 per cent. Further exploration of the mode of action of β-phenethylbiguanide is clearly indicated.

409a Secretion et Utilisation de l'Insuline sous l'Influence du Chlorhydrate de Dimethylbiguanide. J. Sterne (France).

Le diméthylbiguanide agit chez les sujets diabétiques non en stimulant la sécrétion d'insuline comme les sulfonylurées, mais en permettant une meilleure utilisation par l'organisme de l'insuline disponible, même en petite quantité. Chez l'homme ou l'animal pancréatectomisé, il économise les 2/3 des besoins d'insuline.

Aux doses thérapeutiques, dix fois plus faibles que les doses expérimentalement hypoglycémiantes, il ne provoque aucune hypoglycémie chez les sujets normaux, ni chez les diabétiques dont il corrige l'hyperglycémie sans l'abaisser au dessous de la norme, par compensation automatique de l'insulinosécrétion. Au contraire, combiné à de l'insuline exogène, ou à un sulfamide antidiabétique qui stimule aveuglement l'insulinosécrétion, le DMGG peut entraîner de l'hypoglycémie. La combinaison DMGG—sulfamide à longue durée d'action permet l'équilibre de diabètes même avec insulinosécretion résiduelle minime.

Dans les cas de résistance à l'insuline (acromégalie, diabète cortisonique, insulinorésistance des infections chez les diabétiques etc.) l'action facilitante de l'insuline du DMGG est extrêmement intense et rapide.

Certains faits expérimentaux indiquent que le mode d'action du DMGG sur l'insuline se produit au niveau de la membrane cellulaire.

409b Secretion and Utilization of Insulin following Treatment with Dimethylbiguanidine Hydrochloride. J. Sterne (France).

Dimethylbiguanidine acts in diabetics not through stimulation of insulin secretion as for the sulphonylurea compounds, but by allowing better utilization of the insulin available even in small amounts by the body. In animals and man, after removal of the pancreas, there is a saving of two-thirds on the insulin requirements.

Therapeutic doses—ten times less than the hypoglycaemic doses found experimentally—cause no hypoglycaemia in normal subjects nor in diabetics showing it corrects the hyperglycaemia without reducing the level below normal by automatic compensation of the insulin secretion. On the other hand, in combination with exogenous insulin or with an anti-diabetes sulphonamide which indiscriminately stimulates insulin secretion, DMGG can given hypoglycaemia. A combination of DMGG with a long-acting sulphonamide makes equilibrium possible in diabetes even with minimal residual insulin secretion.

In cases of resistance to insulin (acromegalia, cortisone-induced diabetes, insulin resistance in infections in diabetic patients, etc.) the facilitating action on insulin of DMGG is extremely intense and rapid.

Certain experimental findings would indicate that the mode of action of DMGG on insulin occurs at the level of the cell membrane.

410 Effect of Guanidine Derivatives on Oxidative Phosphorylation. B. C. Pressman (U.S.A.).

The hypoglycaemic properties of guanidine and certain of its derivatives have focused attention on their potentialities as antidiabetic drugs. Mitochondrial oxidative phosphorylation has been strongly implicated as the principle point of action of these agents. In order to study the mechanism of action of guanidine in greater depth, a series of alkylated guanidines has been synthesized for the purpose of relating structure to activity. In one test system, measuring the inhibition of respiration by rat liver mitochondria as followed in the oxygen electrode, the most potent of the series tested, decyl guanidine, exhibit 400 x the activity of unsubstituted guanidine. All derivatives examined showed a characteristic time lag and at least partial reversibility by dinitrophenol of the induced inhibition. Another property of guanidine which seems to bear on the inhibition of oxidative phos-

phorylation is the stimulation of mitochondria to bind phosphate from the incubation medium until the total bound phosphate increases 2–3 times that normally found in incubated mitochondria. The ability of the guanidines to inhibit Ca-stimulated ATP-ase parallels their respiratory inhibitory powers, but, in the absence of Ca, the guanidines stimulate endogenous ATP-ase activity. By way of contrast the antibiotic oligomycin, which also induces a dinitrophenol reversible inhibition of respiration, fails to augment phosphate accumulation in mitochondria or raise the endogenous ATP-ase level. It thus appears that the guanidines act by inhibiting the sequence of mitochondrial phosphorylative reactions as at reaction site different from that affected by oligomycin.

411 Decreased Formation of ^{14}C-Glucose from ^{14}C-Lactate after Insulin and Tolbutamide Administration in Diabetics. R. C. DE MEUTTER and W. W. SHREEVE (U.S.A.).

Shortly after the intravenous injection of ^{14}C-DL-lactate in diabetic patients, the radioactive label was found in the blood glucose: 15–30 per cent of the injected dose was calculated to be present in the extracellular glucose, after a tracer dose of lactate or after the infusion of a labelled load of lactate. The amount of ^{14}C incorporated into this biosynthetic glucose thus reflects the activity of the gluconeogenic pathways. This process, presumably mainly a hepatic function, was more active in the untreated diabetics than in non-diabetics.

A significant depression of the amount of ^{14}C converted to glucose was observed in the same patients, when a single tracer dose of ^{14}C-lactate was injected 10–20 min after the intravenous administration of insulin (0·1 unit/kg body wt.) or tolbutamide (40 mg/kg body wt.). This decrease was even more marked when a ^{14}C-lactate load was infused over a period of 60 min, simultaneously with an infusion of insulin. The acute administration of tolbutamide induced a less marked hypoglycaemia than insulin, but the reduction in glucose labelling was of the same magnitude.

Glucose production or release was not interrupted during the hyperglycaemia following a single intravenous injection of glucose (0·33 g/kg body wt.). Analysis of glucose released after injection of glucagon permitted estimations of glycogen labelling.

Differences in the specific activity of the breath $^{14}CO_2$ were also detected after the administration of the hypoglycaemic agents or glucose and will be discussed.

(Supported by the U.S. Atomic Energy Commission.)

412 Further Observations Demonstrating that Insulin, as well as Epinephrine, Increase Hexokinase Activity in Adipose Tissue, in vitro. W. S. LYNN, JR., E. EARNHARDT and R. H. BROWN (U.S.A.).

Previous data from this laboratory have indicated that both insulin and epinephrine markedly increase the quantitative conversion of glucose to lactic acid under strict anaerobic conditions *in vitro*. Under the conditions of the above experiments, tissue glucose concentrations calculated from inulin spaces, urea and H_2O spaces were always less than the concentration of glucose in the buffers. Thus, no conclusions could be drawn as to whether this hormone-induced increase in glycolysis was due to an increase in cellular transport of glucose or due to a more direct stimulation of cellular glycolysis. Experiments, designed to eliminate the necessity of transport of glucose into the cells, were therefore performed. Rat adipose tissue was first pre-incubated in buffer containing glucose, 1,000 mg%, digitonin, 0·3 mg/ml, and hyaluronidase. Under these conditions glucose equilibrates with the same space as urea and water; therefore the intracellular concentration of glucose is 1,000 mg/%. The tissues were then removed from this buffer, and placed in buffer containing the hormones, but no glucose. During the first 5 min in this buffer (during which time the intracellular glucose concentration exceeds that of the buffer) the hormones strikingly increase glycolysis, i.e. lactate production anaerobically or triglyceride and CO_2 synthesis aerobically, despite the fact that the intracellular glucose concentration is much higher than the buffer. Furthermore, extracts of these tissues after the 5 min incubation with the hormones contain more than twice as much hexokinase activity. Thus, these hormones, under these conditions, increase the glycolytic activity of this tissue rather than the transport of glucose. This increase is associated with the appearance of increased amounts of hexokinase in tissue extracts.

413 Effect of Hypoglycaemic Sulphonylurea on the Liver. L. TARDOS and I. ERDÉLY (Hungary).

In previous experiments, it has been shown that if the liver of a 24-hr starved rat is perfused *in vitro* with Krebs–Ringer's phosphate solution, it exudes glucose into the perfusion fluid. The output of glucose was markedly diminished by sulphonylurea. Sulphonylurea was administered *in vivo* and perfusion was made when the maximum hypoglycaemic effect was reached. At the beginning of the perfusion, livers of both treated and control rats contained the same quantity of glycogen. The hepatic glycogen content of treated animals did not differ from that of untreated controls even if glucose had been administered to the animals prior to the experiment and their hepatic glycogen content had been considerably increased. During the perfusion, the glycogen content of the liver decreased. The rate decrease in hepatic glycogen content was the same in both treated and control rats, so there is no marked correlation between hepatic glycogen content and decrease of glucose output caused by sulphonylurea. The cause of decreased glucose output is connected with increased utilization of glucose by the liver. This is confirmed by the fact that the output of lactic acid decreased parallel

with that of glucose. Thus glucose derived from glycogen is oxidized by liver cells to an increased extent. Insulin exerts similar effects and one may suppose that the increase of glucose utilization in the liver caused by sulphonylurea is mediated by insulin.

414 Insulin Potentiation by Tolbutamide in Eviscerated Animals. J. MADSEN (Denmark).

Eviscerated, nephrectomized cats were given glucose intravenously at a constant rate in an amount sufficient to keep the plasma glucose concentration nearly constant.

After a control period of 2 hr, one group of these animals was injected intravenously with tolbutamide 100 mg/kg + insulin 0·01 unit/kg. A second group received only insulin. A marked (and statistically significant) difference was found between the hypoglycaemic response in the two groups, hypoglycaemia being most pronounced in the first group (25 per cent compared to 10 per cent in the second group).

When tolbutamide was given without insulin to glucose-infused, eviscerated cats, a slight, statistically insignificant effect was found by comparison with a group injected with saline instead of tolbutamide. This effect was even more insignificant if the animals had been pancreatectomized 2 days before evisceration.

These results were interpreted as showing a "potentiation" of insulin by tolbutamide. The effect seen in animals not given insulin is probably due to potentiation of small amounts of endogenous insulin remaining in the animals from before evisceration.

This insulin potentiation might be explained by an action of tolbutamide on the insulin molecule. However, this hypothesis was not sustained by experiments showing that the hypoglycaemic effect of insulin incubated with minute amounts of tolbutamide was not more pronounced than the effect of the same amounts of insulin and tolbutamide injected separately.

415 Changes in Glucose Concentration in the Blood of the Femoral Artery and of the Hepatic, Portal, and Femoral Vein during Onset of Tolbutamide- or Insulin-hypoglycaemia. A. HASSELBLATT (Germany).

Sulphonylureasy have well-established effects on tissues other than the islets of Langherans, especially on the liver. The possible bearing of such extrapancreatic actions for the hypoglycaemic response, however, is poorly understood as long as hepatic effects of insulin, liberated into the portal vein and thus reaching the liver directly, are still subject to discussion. In the present experiments, an intravenous infusion of tolbutamide has been compared to an infusion of insulin into the portal vein. Blood samples have been withdrawn simultaneously from the femoral artery and the femoral, hepatic, and portal veins using indwelling cannulas. Throughout a two-hour infusion of tolbutamide, the reduction in glucose concentration in the hepatic vein exceeded corresponding changes observed in arterial and portal blood. The difference in glucose concentration between femoral artery and femoral vein was scarcely affected by tolbutamide. Apparently, tolbutamide-hypoglycaemia is primarily achieved by a reduction of hepatic glucose-release. An infusion of insulin into the portal vein produced initially similar changes. During the first 30 min of the experiment glucose concentration in the hepatic vein was markedly reduced as compared to arterial and portal values. Subsequently, this effect of insulin disappeared and in the second hour of the infusion a steady increase of the glucose gradient between femoral artery and femoral vein indicated an increased uptake of glucose by the muscular tissues of the hind leg. Insulin-hypoglycaemia is therefore only initially achieved by glucose retension in the liver, while later on glucose utilization by peripheral tissues is enhanced.

416 Mode of Action of Antidiabetic Drugs. S. OHASHI, S. TAKEUCHI and M. TOBE (Japan).

It was established by us that continuous infusion of mesoxalic acid into the pancreatico-duodenal artery results in hypoglycaemia. At the same time, it was demonstrated by means of the rat-hemi-diaphragm method and experiments with hypophysectomized and adrenalectomized rats that hypoglycaemia brings about an increase of an insulin-like substance in serum.

In hepatectomized dogs under continuous infusion of glucose, sulphonylurea compounds act to produce hypoglycaemia, but in pancreatectomized and hepato-pancreatectomized dogs, sulphonylurea does not cause a fall in blood sugar level.

In normal dogs, phenethylbiguanide acts to produce hyperglycaemia but not methylbiguanide. In adrenalectomized dogs administration of biguanide compounds did not affect blood sugar level which remained normal. In hepatectomized dogs under continuous infusion of 250 mg/kg/hr of glucose, it was established by us that the action of biguanide results in hypoglycaemia. It must be noted here that in the actions of biguanide and sulphonylurea differ in that in hepato-pancreatectomized dogs administration of biguanide causes fall in blood sugar level.

417 The Effect of Strophanthin G (Ouabain) on the Potential and Short-Circuit Current of the Isolated Skin of Frogs and Toads. W. F. WIDDAS (United Kingdom).

The potential and short-circuit current of isolated amphibian skins were measured at 10-min intervals before and after the administration of strophanthin G in an apparatus similar to that described by

Ussing.[1] With isolated frog skin (*rana temporania*) there was a decrease in potential and short-circuit current following the administration of the drug which, after a latent period, was nearly linear until the value had fallen to about half the initial value.[2] The time for the reduction of the short-circuit current to half of its initial value ($T_{\frac{1}{2}}$) depends on the dose of strophanthin G and the potassium concentration of the outside fluid. Skins previously stimulated with pitressin (0·2 unit/cm²) behaved similarly. With a constant potassium concentration of 2·7 mM and 20°C. $T_{\frac{1}{2}}$ varied from about 100 min for 2×10^{-7} w/v strophanthin G to 15 min for 10^{-6} strophanthin G.

Isolated toad skin (*bufo bufo*) was unaffected by strophanthin G up to concentrations of 10^{-6} w/v but a partial fall off of potential and short-circuit current was observed at 5×10^{-6} w/v ($T_{\frac{1}{2}} = 50$–80) min. Frog skins are thus approximately one hundredfold more sensitive to strophanthin G than toad skins.

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418 Some Actions of Cardiac Glycosides on the Frog Striated Muscle Membrane. M. H. Draper, H. Friebel and K. Karzel (United Kingdom and Germany).

Local anaesthetics such as cocaine, procaine and xylocaine (in doses of the order of 1 mM) have been shown to produce a progressive slow reversible reduction in the resting potential of frog sartorius muscle technique, the cardiac glycosides, G-strophanthin, digitoxin and lanatoside A, have also been be antagonized by Ca^{++}.[2] Using the same paired muscle technique, the cardiac glycosides, G-strophanthin, digitoxin and lanatoside A, have also been found to have a membrane depolarizing action. Strophanthin was effective in doses of the order of 10^{-7} M and the more polar lanatoside of the order of 10^{-8} M. The membrane depolarizing action differed from that produced by the local anaesthetics in that it usually took 2 hr for any effect to occur and the subsequent development of the 10–15 mV depolarization was not influenced by attempts to wash out the drug. Reversibility was not observed up to 5 hr after removal of the drug. Furthermore, the presence of Ca^{++} appeared necessary for the production of this depolarizing action. As opposed to the results obtained from local anaesthetics, the action potential had essentially the same appearance as those in the untreated companion muscle. Furthermore, there was a demonstrable gain in Na^+ and Cl^- and loss of K^+ in the treated muscles, but these ionic changes are too small to account for the membrane potential reduction. These ionic changes can be explained by an interference with the Na^+ and K^+ pump mechanism in the cell membrane.[3]

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419 Membrane Activities of Single Myometrial Cells and Progesterone Block. A. Csapo and H. Kuriyama (U.S.A.).

The membrane potential of the pregnant uterus increases during gestation. Placental sites show higher membrane potentials than the non-placental. Rabbit myometrium during gestation generally shows no spontaneous discharge and no response to oxytocin except in the last stages of pregnancy; the mouse and rat myometria can generate spontaneous discharges and respond to oxytocin earlier than the rabbit uterus. Membrane activities are more irregular in the pregnant than the postpartum uterus.

The threshold of excitability in pregnant myometrium is higher. However, the pregnant myometrium is less susceptible to block by depolarization than is the postpartum myometrium. In early pregnancy, propagation of contraction in response to stimulation at one end of a strip is blocked; in late pregnancy it is partially blocked, and in the postpartum myometrium it is propagated. In a uterus at the thirtieth day of pregnancy, tested 24 hr after ablation of the placenta, the contraction was better propagated than in a control.

In vivo treatment with progesterone increases the membrane potential, but less than does treatment with oestrogen. On the last day of gestation in the rat, progesterone prolongs pregnancy and decreases membrane activity.

In vitro treatment of the myometrium with progesterone inhibits spike generation and the development of tension. The block of tension development takes longer in the pregnant than in the postpartum uterus. Block of spike propagation precedes hyperpolarization and also precedes block of pacemaker activity.

These observations indicate that the maintenance of pregnancy until delivery may be due to the presence of progesterone which suppresses membrane activity.

420 Drug-Induced Changes in the Permeability of Isolated Mesentery. R. E. Gosselin and W. O. Berndt (U.S.A.).

Equations of passive diffusion describe adequately the passage of ^{86}Rb cations through isolated rabbit mesentery in the absence of electrochemical and hydrostatic pressure gradients.[1] A double-tracer technique has been employed to extend these

observations to creatinine (chemically determined) and to ^{32}P orthophosphate, each of which was measured at the same time as the ^{86}Rb flux. For ^{32}P as well as for ^{86}Rb, the kinetics of passive diffusion were demonstrated; the same was assumed to be true for creatinine. Permeability constants (K) determined at 38°C averaged 0·042, 0·026 and 0·022 cm/min for ^{86}Rb, creatinine, and ^{32}P respectively. Drugs and changes in temperature influenced differentially the permeability of mesentery to these three solutes. Between 38° and 28° C the K for ^{32}P decreased most and for ^{86}Rb least (Q_{10}= 2·1 for ^{32}P, and 1·6 for ^{86}Rb). Histamine increased the permeability to both ions, to ^{86}Rb more than to ^{32}P. With 5-hydroxytryptamine the K for ^{86}Rb rose markedly but the constant for ^{32}P was unaffected or even fell. Conversely, the antidiuretic hormone increased the ^{32}P flux while reducing the ^{86}Rb flux.

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421 Effect of Certain Drugs on the Odontoblast Membrane Potential. D. C. Kroeger (U.S.A.).

The odontoblast with its fine protoplasmic process extending through the dentin of the tooth has been proposed as being a secretory cell by some workers and a sensory cell by others. To understand more fully the physiology of this cell, we have measured its membrane potential using ultramicroelectrode techniques combined with the application of both labilizer and stabilizer compounds. Odontoblasts grown in tissue culture as well as *in vivo* cells in the teeth of young rats and dogs have been studied.

The odontoblast in tissue culture or *in vivo* possesses a resting membrane potential of 35–40 millivolts similar to that reported for smooth muscle and glandular epithelium. Stimulation of the peripheral end of the mandibular nerve in rats or the application of acetylcholine (10^{-7} moles) to the perfusion medium causes a hyperpolarization of the membrane potential of approximately 3–4 millivolts lasting 15–25 sec. Epinephrine, histamine, and 5-hydroxytryptamine have not been observed to cause this effect. Atropin appears to prevent this transient hyperpolarization as does the local anaesthetic, procaine.

In view of the controversy concerning the embryological origin of the odontoblast, these findings tend to support the concept of an ectodermal origin of this cell. These studies do not permit a conclusion as to whether the cell is sensory or glandular in function.

422 Effects of Sodium Diatrizoate and Sodium Acetrizoate on the Blood-Brain Barrier. J. O. Hoppe, A. Brousseau and E. W. McChesney (U.S.A.).

The use of iodinated, water-soluble organic compounds as roentgenographic diagnostic aids is occasionally associated with minor systemic reactions and, rarely, with fatal reactions. It has been suggested that these rare fatal reactions in man may arise from an intrinsic susceptibility to breakdown of the blood–brain barrier.[1] Evidence to be discussed in support of this hypothesis is derived from the following observations on Na diatrizoate and Na acetrizoate in the rabbit: (a) the acute intravenous/intracisternal LD_{50} ratios were found to be 1,452 and 658, respectively, in contrast to a ratio of 7·5 for a known centrally acting substance such as chlorpromazine; (b) the slopes of the dose–mortality curves by both routes of administration were similar; (c) the convulsions in the lethal dose range were observed by both routes whereas pulmonary vascular changes were observed only after intravenous injection; (d) anaesthesia reduced the acute intracisternal toxicity of both compounds; and (e) Na acetrizoate produced extensive breakdown of the blood–brain barrier whereas Na diatrizoate produced only minimal changes. Most of these observations have also been confirmed in the cat and dog. Cerebrospinal fluid–iodine levels with Na acetrizoate were found to be 10–100 times greater than those observed with Na diatrizoate following rapid intra-carotid injection of 50 per cent solutions of both compounds in the pentobartitalized dog. These data indicate that differences in chemical structure and penetrability of the blood–brain barrier provide a firm pharmacological basis for the source of these rare fatal reactions.

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423 Changes in Yeast Permeability Induced by Nystatin. J. O. Lampen (U.S.A.).

The polyenic antifungal antibiotic nystatin is bound rapidly by log-phase cells of *Saccharomyces cerevisiae* LK2G12, by protoplasts, and by the membrane fraction obtained following osmotic lysis. A rapid, thus far irreversible swelling of the protoplasts ensues. There is no significant activation of the external ATPase. At low concentrations of nystatin (0·5–5 μg/ml) the cellular K^+ and a part of the NH_4^+ are rapidly lost. At neutral pH the resulting inhibition of glycolysis (log-phase cells) can be prevented or reversed by K^+—NH_4^+ mixtures. With 10–60 μg of nystatin/ml, CO_2 production (20–30 per cent of the original rate) occurs only in the presence of K^+—NH_4^+, Mg^{++}, AMP (or ATP), DPN, thiamine pyrophosphate, and a primer. High levels of NH_4^+ stimulate CO_2 production beyond that obtained with excess K^+. Anaerobic decarboxylation of pyruvate is activated, and an increased loss of organic acids, sugar phosphates, and nucleotides occurs. Some loss of protein is occasionally observed, but the glycolytic activity remains with the treated cells even after washing. These cells probably still retain selective permeability barriers since they do not produce CO_2 from

glucose 6-phosphate or fructose diphosphate. The permeability alterations in these cells and the possible formation of CO_2 from glucose via the hexose monophosphate shunt will be discussed.

424 Role of C-Factor in Water Uptake and Extrusion by Mitochondria and Interference by Various Drugs. D. Neubert and A. L. Lehninger (U.S.A.).

Water uptake by rat liver mitochondria in a KCl-Tris-medium caused by thyroxine, inorganic phosphate, Ca^{++} and under certain circumstances by organic mercurials and -SH or -SS- compounds may be reversed by adding ATP, Mg^{++} and serum albumin to the test system.[1] However, an additional factor (C-factor) is required to contract mitochondria swollen with 0·01–0·03 M glutathione (GSH). GSH causes "leakage" of C-factor, which must be restored to observe water extrusion.[2]

This membrane component, solubilized by sonic oscillation, has been purified considerably by selective heat denaturation, isoelectric precipitation, ethanol fractionation and chromatography on DEAE- or CM-cellulose. Its action is rather specific; other proteins and enzymes show no activity under the conditions tested.

Data on the biochemical characteristics of this factor and on the influence of various drugs and enzyme inhibitors are presented. The significance of C-factor for water uptake and extrusion by mitochondria and a possible role of this or similar factors in movements of water through other membranes as well as a possible connexion to respiratory and phosphorylating enzymes is discussed.

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425a Modifications de la Charge Electrique de Surface au Cours d'Actions Pharmacologiques sur la Perméabilité Cellulaire. (Etude par des Méthodes Électrocinétiques.) P. Rossignol et C. Pattani (France).

Selon les conceptions actuelles, les modifications pharmacologiques de la perméabilité concernent surtout la cinétique des échanges ioniques lors de l'activité électrique. (Notions de "stabilisateurs" et de "labilisateurs" de la membrane.)

Le potentiel de membrane, cependant en rapport direct avec les gradients ioniques, se montre insuffisant, même lorsqu'il est nettement influencé, pour rendre compte des variations de perméabilité. La charge électrique de surface diffère du potentiel de membrane; c'est une polarisation structurale qui intéresse la couche la plus externe de la membrane et résulte de l'orientation de molécules polaires. Non mesurable directement, elle doit être évaluée par l'intermédiaire de phénomènes électrocinétiques.

Nous avons recherché l'influence de quelques agents pharmacologiques sur le débit de liquide traversant par électroosmose une oreillette de lapin. La procaïne (40 mg/100 ml, dose provoquant le bloc de l'oreillette isolée battante) diminue nettement le flux électroosmotique. La quinidine agit de même. L'acétylcholine au contraire augmente le flux, mais de façon transitoire et non parallèle à son action pharmacologique car cette accélération n'est pas toujours immédiate et demande parfois dix minutes. L'atropine, aux concentrations habituellement inhibitrices de l'acetylcholine, reste sans effet. La procaïne et l' l'acétylcholine n'agissent pas sur le flux électroosmotique à travers une couche de silice.

Les concentrations utilisées ne peuvent modifier la constante diélectrique, la viscosité et l'écartement de la double couche.

Dans nos expériences, la seule variable est donc la charge de surface qui pourrait constituer ainsi un des facteurs de la régulation de la perméabilité.

425b Changes in Surface Electric Charge During Action on Cell Permeability of Pharmacological Substances (Electrokinetic Study). P. Rossignol and C. Pattani (France).

In line with present ideas, pharmacological changes in permeability chiefly concern the kinetics of ion exchange during electrical activity (the conception of "stabilizers" and "labilizers" of the membrane).

The membrane potential, though directly related to the ionic gradients is insufficient even when plainly influenced to reflect the variations in permeability. The electrical charge on the surface differs from the potential of the membrane: it is a structural polarization which concerns the outermost membrane layer and results from orientation of the polar molecules. As it cannot be measured directly, it must be evaluated from electrokinetic phenomena.

We have investigated the effect of certain pharmacological substances on the flow of liquid through the rabbit auricle by electro-osmosis. Procaine (40 mg/100 ml, a dose causing blockade of the isolated beating auricle) appreciably reduces the electro-osmotic flux. Quinidine acts in like manner. Acetylcholine, in contrast, increases the flux but only briefly and not in parallel with its pharmacological action since this acceleration is not always immediate, sometimes requiring 10 min. Atropine at concentrations usually inhibiting acetylcholine is ineffective. Procaine and acetylcholine do not act on the electro-osmotic flow through a layer of silica.

The concentrations used do not change the dielectric constant, viscosity or distance between the double layer.

In our experiments the only variable was the charge on the surface, which would therefore appear to be a factor in the regulation of permeability.

426 Studies on the Mechanism of Action of TSH in Thyroid Iodide Transport. D. M. Woodbury, R. Karler and G. Heninger (U.S.A.).

Studies in normal, TSH-treated, and hypophysectomized rats indicate that the thyroid follicular fluid contains more Na^+ and HCO^-_3 than extracellular fluid whereas follicular cells contain normal concentration of K^+. Hypophysectomy, by increasing follicular fluid volume and decreasing cell volume, markedly increases Na^+ and Cl^- and markedly decreases water and K^+ concentrations in the thyroid. TSH, by decreasing follicular fluid volume and increasing cell volume, produces opposite effects. HCO^-_3 appears to concentrate in follicular fluid; hypophysectomy increases HCO^-_3 concentration, and decreases iodide concentrating ability; TSH and acetazolamide decrease HCO^-_3 concentration and increase iodide concentrating ability. Thus, HCO^-_3 concentration appears to be inversely proportional to iodide concentration. Since HCO^-_3 concentration is intimately related to carbonic anhydrase (C.A.) activity in other tissues, C.A. activity of the thyroid was measured in different functional states. C.A. activity in follicular cells was decreased by TSH and acetazolamide, by propylthiouracil in intact but not hypophysectomized rats, and by chronic iodide deficiency; all these procedures increase thyroid iodide uptake. These data suggest that HCO^-_3 normally competes with iodide for the cellular carrier system which actively transports anions into follicular fluid. HCO^-_3 originates from metabolically produced CO_2 and its concentration is regulated by C.A. TSH and acetazolamide inhibit C.A., thereby decreasing HCO_3 concentration. Therefore, less HCO^-_3 is available to compete with iodide for the transport system; hence, iodide uptake is increased. Hypophysectomy produces effects opposite to those of TSH and acetazolamide.

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427 The Effect of Orphenadrine (Disipal) on Cell Membrane Permeability. M. J. E. Ernsting, W. F. Kafoe, W. T. Nauta, H. K. Oosterhuis, P. A. Roukema and C. de Waart (Holland).

The inhibition by orphenadrine of the oxidative metabolism of brain tissue, metabolizing *in vitro*, depends on the concentration of the inhibitor. The amino acid level in the incubation fluid was found to rise more or less parallel with the inhibition. This influence on amino acid metabolism may, among other things, be based on a change in the cell membrane permeability as a result of interference with the energy supply. Shifts in Na and K concentrations point in this direction.

We investigated whether these phenomena are connected with ATP formation and whether the permeability for certain dye-stuffs changes accordingly.

428 Decarboxylation of o-Tyrosine and 5-Hydroxytryptophan by Tissues of Rats Treated with Cortisone and Thyroxine. V. E. Davis, C. Hanly and J. Awapara (U.S.A.).

Most tissues of the rat can decarboxylate L-*o*-tyrosine and L-5-hydroxytryptophan. The levels of these activities are somewhat dependent on the concentration of pyridoxal phosphate.

Experiments were performed to test the role of cortisone and thyroxine on the decarboxylation of *o*-tyrosine and 5-hydroxytryptophan by various organs of the rat. Male rats (250–350 g) were treated with cortisone (30 mg/kg) for 10 days. Another group was treated with thyroxine (2·5 mg/kg) for 16 days. Other groups were (a) sham-operated, (b) adrenalectomized, (c) exposed to 4°C, and (d) treated with desoxycorticosterone acetate (10 mg/kg) for 10 days.

The rate of decarboxylation of these two substrates increased up to 60 per cent in the livers of rats treated with cortisone; however, no change of decarboxylase activity was observed in the kidneys or brains. A decrease of 70 per cent in the activity with *o*-tyrosine as substrate was noted in the livers of rats treated with thyroxine. A decrease in this activity was also observed in the brains of the animals from this group. Livers of adrenalectomized animals given de-ionized water showed a decrease in the rate of decarboxylation of these substrates. Desoxycorticosterone acetate was without effect.

The action of cortisone on the decarboxylation of *o*-tyrosine and 5-hydroxytrytophan seems to be related to changes in the apoenzyme. The action of thyroxine is now under study.

429 Inhibitory Action of Steroids on Anterior Pituitary Functions. G. Gavazzi, G. Giuliani, G. Mangili and L. Martini (Italy).

The action of steroids on the release of ACTH and gonadotrophic hormones has been studied in rats. The following procedures were employed: (a) block of adrenal ascorbic acid depletion in the remaining adrenal 1½ hr after unilateral adrenalectomy; (b) drop of blood corticosterone levels as measured by the method of Silber[1] as modified by Guillemin[2]; (c) block of urinary gonadotrophins output in ovariectomized rats[3]; and (d) decrease of blood LH titres in ovariectomized animals; LH was assayed by the ovarian ascorbic acid method in recipient heavily luteinized rats.[4]

In the study of ACTH block, the potency of the adrenocortical steroids was as follows: cortisone < hydrocortisone = triamcinolone < prednisone < medrol < 9α-fluorohydrocortisone < prednisolone < Δ′ 6α-chlorohydrocortisone < oxylone < dexamethasone.

Steroids with little or no adrenocortical activity were also found active as ACTH blockers. Total gonadotrophins and LH release inhibition was

obtained with progesterone and testosterone-like compounds.

1. Silber (1958), *Chim. Clin.*, **4,** 278.
2. Guillemin (1959), *J. Lab. Clin. Med.*, **53,** 830.
3. Mangili (1960), *Atti Accad. Med. Lomb.*, **15,** 55.
4. Giuliani (1961), *Acta Endocr.* In press.

430 The *in vitro* Responsiveness to ACTH of the Adrenals of Rats Treated with Various Corticosteroids. G. Fekete and P. Görög (Hungary).

In our preceding publications, the alteration of the adrenal responsiveness to ACTH in rats by various hormones has been demonstrated by the Sayers' test. In the present experiments, we studied the steroidogenetic capacity of the adrenal gland to react to a standard quantity of ACTH after previous treatment of the rats with various corticosteroids.

The therapeutically equivalent quantities (ratio = 33 : 6, 6 : 1) of cortisone, prednisolone and dexamethasone were given to the rats for 6 days in two doses. On the seventh day, the 17-hydroxysteroid producing capacity of the adrenals was determined by the action of 9 and 27 milliunits inherent in the parent Δ^4-3-ketone; actually, by ACTH, respectively. The absolute value of the difference in the steroid production brought about by the two doses of ACTH served as a base to the evaluation.

Steroid production was mostly blocked by cortisone; considering the adequate rate of doses, however, there was but a slight difference between the action of the three compounds. The difference between the slopes of the three curves was similarly small, that of the dexamethasone group being the most precipitous. This indicates that overdosage with dexamethasone involves the greatest danger with regard to adrenal hypofunction.

The same phenomenon seems to be confirmed by our present experiments aimed at examining the *in vitro* direct blocking action of the same steroids.

431 Inhibition of 17-α-Hydroxylation of Adrenal Steroids. H. Sheppard and J. J. Chart (U.S.A.).

Previous studies designed to detect compounds which would inhibit the biosynthesis of adrenal steroids have led to the availability of Metopirone, Su-4885. This compound, 2-methyl-1:2-bis-(3-pyridyl)-1-propanone, when used at clinically tolerable doses, acts like an inhibitor of 11-β-hydroxylation and results in an increased production of 11-desoxy cortisol and 11-desoxy corticosterone. Recent work has uncovered two compounds which behave like inhibitors of 17-α-hydroxylation and result in a decrease in cortisol and an increase in corticosterone production. Both compounds, Su-8000, 3-(6-chloro-3-methyl-2-indenyl)-pyridine, and Su-9055, 3-(1:2:3:4: tetrahydro-1-oxo-2-naphthyl)-pyridine, are active in dogs having acutely cannulated adrenal veins. The increased production of 11-desoxycortisol which occurs in the dog after Su-4885 may be inhibited by subsequent administration of Su-9055. Likewise, the increased production of corticosterone which occurs in the dog after Su-9055 may be inhibited by subsequent administration of Su-4885. These results would be expected from the combined usage of inhibitors of 11-β and 17-α-hydroxylation. Some of these effects have been demonstrated *in vitro* with isolated adrenal glands incubated with radioactive progesterone and will be discussed in detail.

432 Qualitative and Quantitative Changes Brought about in the Biological Activities of Steroids by Esterification or Etherification. A. Ercoli (Italy).

The degree of biological activity of hormonal steroids given parenterally may be enhanced or decreased to a notable extent through esterification with suitable organic acids. Esterification, however, does not appear to affect considerably the quality of the hormone activity. Rather different is the behaviour of steroidal ethers, about which the following statements may be made:

(a) Enol ethers of Δ^4-3-ketosteroid hormones, formed by condensation with alcohols of suitable carbon chain length, are more active by mouth than either the same compounds given parenterally or the parent ketones given orally.

(b) Such a potentiation is not necessarily shared to the same extent by the various hormonal properties inherent in the parent Δ^4-3-ketone; actually, by using a convenient alcoholic carbon chain, it is possible to obtain a clear-cut dissociation of activities.

(c) Given parenterally, enol ethers of Δ^4-3-ketosteroid hormones show reduced or abolished hormonal properties.

(d) Such a reduction does not necessarily affect certain properties which would be better thought of as pharmacological. These are, indeed, markedly influenced by the length of the alcoholic carbon chain.

(e) The aforementioned behaviour may be seen also in non-enolic steroidal ethers.

It appears likely that the carbon chain tied up to the steroid molecule with an etheric linkage influences not only the absorption processes, but also some steps of its metabolic pathway.

433 The Neurogenic Influence of Dogs and Cats on the Corticosteroid Content in Rat Plasma and the Excretion of Corticosteroids in Guinea Pigs' Urine. A. Pekkarinen, E. Tala, E. Sotaniemi and N. Niemelä (Finland).

The effect of environmental and emotional factors and blood sampling upon the content of

corticosteroids in the plasma of rats have been elucidated. A fluorimetric micromodification has been developed for the analysis of 0·1 ml rat plasma, collected from the tail vein during Fluothane anaesthesia and using a small dose of dexamethasone (0·01–0·02 mg/kg) as an inhibitor of the hypophyseal adrenocortical stimulation during collection of blood samples. For the studh of environmental factors, the rats were kept in cages close to dogs or cats for 1–2 min to 3 hr, during which time the dogs made a considerable noise. During the strongest neurogenic influence the rats were almost without activity and motionless. The neurogenic stimuli clearly increased the content of corticosteroids in the plasma in a few minutes. The increase was observed clearly during the first hour. A small dexamethasone dose, 0·01–0·02 mg/kg, prevented the rise caused by the blood sampling, but did not prevent the rise caused by the neurogenic timulus. A larger dexamethasone dose, however, 0·1–1·0 mg/kg, can prevent the effect of neurogenic strain. The neurogenic rise of corticosteroid content could be inhibited by gradually increasing doses of dexamethasone, either partially or totally. Dexamethasone may thus be able to influence either humorally or through the brain the regulation of ACTH secretion. The tape recorded sound of barking dogs or a timed bell and light also increased the corticosteroid content. Audiometric sounds were not normally effective.

The influence of neurogenic strain upon the excretion of corticosteroids has been studied in guinea pigs in the dog room and in the control group in the quiet room. The 24-hr excretion of total 17-hydroxycorticosteroids has been analysed according to Jenkins *et al.* method (1955). The neurogenic stimuli in the dog room increased the excretion of total 17-hydroxycorticosteroids in guinea pigs. The excretion reached its maximum in the dog room in one week. The urine volume was also increased.

434 The Neurogenic Influence of Dogs and Cats on the Corticosteroid Content in Rat Plasma and the Excretion of Corticosteroids in Guinea Pigs' Urine. The Effect of Psychopharmaca and Anaesthetic. A. Pekkarinen, E. Tala, E. Sotaniemi and N. Niemelä (Finland).

The effect of psychopharmaca, psychosedative and neuroleptic drugs upon the content of corticosteroids in rat plasma has been elucidated. The content of corticosteroids was analysed in 0·1 ml rats plasma by the micromodification of the fluorimetric method of estimating corticosteroids (Pekkarinen and Tala). The samples were collected during Fluothane anaesthesia after injection of a small dose of dexamethasone, which inhibits the increase of content, which normally occurs during the blood sampling. The increase of corticosteroid content which follows blood sampling, cannot be prevented by local anaesthetic or pentobarbitone. Combination of morphine with pentobarbitone can partially inhibit this increase of corticosteroid content.

The effect of psychopharmaca upon the content of corticosteroids in rat plasma was studied in quiet conditions and under neurogenic stimulation by dogs on the humoral or neural pathways of ACTH release and to evaluate the possibility of using the neurogenic increase of corticosteroids as a psychopharmacological test method. Psychosedatives, e.g. meprobamate could not effectively inhibit the neurogenic increase of corticosteroid content, although they could partly diminish it. In quiet conditions after psychopharmaca, the basal content was normal. Chlorpromazine in a large neuroleptic dose prevented the rise in the content of corticosteroid content in the dog room, but not in small doses either in the dog room or cat room. After chlorpromazine and promazine, the basal content in the quiet room was increased somewhat above normal. Other neuroleptic drugs (Stemetil, Trilafon, Truxal, reserpine) were also not very effective in inhibiting the neurogenic increase of corticosteroids in the dosage used. However, the average content was somewhat lower under neurogenic influence of cats than in the control group without neuroleptic drugs.

Anaesthetics, e.g. ethyl ether, Fluothane, ethyl chloride and Vinesthene increased the corticosteroid content without dexamethasone treatment. A small dose of dexamethasone controls the increase of corticosteroid content in Fluothane anaesthesia and partly in ether anaesthesia. A large dexamethasone dose is needed to control the increase of corticosteroid content in ethyl chloride or Vinesthene anaesthesia.

Psychopharmaca, e.g. meprobamate, reserpine and chlorpromazine could not decrease the neurogenic increase of corticosteroid excretion in guinea pigs urine in the dog room. Iproniazide did not rise the excretion of corticosteroids higher than its neurogenic control. High dose of hydroxyzine had a tendency partly to decrease the neurogenic increase of corticosteroid excretion, but smaller dose was not effective.

435 Some Pharmacological Properties of Δ^4-β-norandrostane-3:17-dione. I. Janků, R. Smetana and J. Joska (Czechoslovakia).

The experiments of Selye *et al.* demonstrated central depressive effects of various steroid substances when administered intraperitoneally to rats in high dosage.

In connexion with the synthesis of some β-nor steroid derivatives we studied some pharmacological properties of Δ^4-βnorandrostane-3:17:dione, which according to our findings possesses a weaker anabolic and androgenic activity in comparison with Δ^4-androstane-3:17-dione. After intraperitoneal administration of 100–200 mg/kg of the

compound in mice, we observed convulsions of a clonic–tonic type followed by a stage of general depression. These phenomena were accompanied by extensive mydriasis and by a decrease of propulsive motility of the small intestine. At the same time thiopentone narcosis was significantly prolonged whereas during depression a marked protection against lethal effects of leptazole occurred. Δ^4-Androstane-3:17-dione was also active, but to a lesser extent. In rats, convulsions were less frequent, but the dose of 200 mg/kg i.p. caused a complete inhibition of dextran-induced oedema. Further investigations showed a long lasting hyperglycaemia and a depletion of muscle glycogen following doses of 100–200 mg/kg. In adrenalectomized rats, we observed a deposition of glycogen in the liver, as well as a decrease of diuresis with a high retention of Na^+ and K^+ after 100 mg/kg, a double dose being lethal for the majority of animals. These results together with previous findings of this laboratory point out to the fact that inactive steroid compounds or those with minor physiological activity may cause profound metabolic alteration when injected intraperitoneally in high dosage. It is suggested that these changes should be taken into account when explaining the mechanism of central effects of steroids observed under such conditions by Selye and other workers.

436 On the Mechanism of the Adrenal Cortex Stimulation by Reserpine. N. Canal, G. Giuliani, A. Maffei-Faccioli and T. Scamazzo (Italy).

The mechanism of adrenal cortex stimulation in the rat by reserpine has been studied using the tests of haepatic tryptophan peroxidase induction and the blood levels of corticosterone.

Amine oxidase blocking drugs inhibit the adrenal stimulation while imipramine, in spite of reversing the sedative effects of reserpine, does not.

Also, adrenolytic drugs (ergotamine, phentolamine, dibenamine), ganglionic blocking drugs (pentolinium), and drugs which suppress the cortical (SKF-3195A) and the medullary (P-236) function, cannot antagonize the effects of reserpine on the adrenal cortex.

These results are in favour of the hypothesis that reserpine brings about adrenal stimulation inducing ACTH release, either by a direct mechanism or by activating a hypotalamic centre.

Friday. August 25

437 Pharmacology of Respiratory Neurones. G. C. Salmoiraghi (U.S.A.).

A system of nerve cells, the respiratory neurones, dispersed among non-respiratory units throughout the lateral medullary reticular system, is essential to the control and maintenance of rhythmic breathing. There appear to be only two functional classes of respiratory neurones: inspiratory and expiratory. There is no evidence that any of these neurones could fire spontaneously if isolated from all activity in neighbouring nerve cells; rather, it appears that respiratory neurones can discharge periodically only when facilitated by other systems. Synchrony of burst activity among cells of the inspiratory or expiratory systems appears to be maintained by functional connections between units of the same population. It seems probable that such inter-connections provide paths for self-re-excitation within the networks of inspiratory and expiratory neurones. Reciprocal innervation between the inspiratory and expiratory neural systems and increase in the firing threshold of inspiratory and expiratory units as the burst of action potentials progresses appear to play a major role in periodically bringing the discharge of such units to an end.

Against this background of information regarding the organization of the brain-stem neuronal organization that controls respiration, the central effects of pharmacological agents reported to induce central depression or excitation of respiration will be analyzed and discussed.

438 Drug Induced Patterns of Motor Activity. W. Feldberg (United Kingdom).

This paper is a review of recent experiments on tremor, scratching and convulsive activity produced by drugs acting from the inner or outer surface of the brain. The sites of action and some of the changes produced by these drugs on the electrical activity of the cerebral cortex are discussed.

439 Drug-Induced Changes in Behaviour. E. Jacobsen (Denmark).

If an animal is exposed to a certain situation (e.g. a dog is shown to a cat), it will behave in a manner which is characteristic for the species and the individual. This behaviour is normal for the animal in this special situation, but it may happen that the same animal under the influence of a drug reacts differently in a way which might be normal in other situations but is absolutely abnormal in this special situation. Such changes are symptoms of the effect of the drugs on the highest measurable level of the C.N.S. Observations and descriptions of the drug-induced behavioural changes are essential in the study of psychotropic drugs.

However, the effect of drugs on animal behaviour is difficult to evaluate. Our knowledge of normal animal behaviour is still limited. Another difficulty is that most psychotropic drugs also have an effect on lower centres of the C.N.S. and may cause muscular inco-ordination, rigidity, convulsions, anaesthesia, etc., masking possible behavioural changes.

The various types of psychotropic drugs induce typical and consistent behavioural changes on which a grouping can be based. This grouping differs in some points from the grouping based on other criteria, but is in conformity with them in others.

Further study of drug-induced behavioural changes must primarily be designed to seek for localized biochemical or electrical changes after administration of the drugs, followed by a comparison with symptoms caused by local irritation or ablation of parts of the C.N.S. of normal animals. In this way information about the site of action of the drug may be obtained. A comparative analysis of the effect of psychiatric symptoms may perhaps give information about the nature of the disease favourably influenced by the drugs. The possibilities and difficulties of these programmes will be discussed.

440 Pharmacological Modifications of Social Behaviour. J. M. R. Delgado (U.S.A.).

Clinical improvement of social behaviour is one of the main objectives of psycho-pharmacology. However, little experimental work has been done on the influence of drugs on social behaviour, and this is the purpose of the present communication.

Two methods, which may be applicable to other types of investigations have been developed: (1) The behaviour of a colony of 6 monkeys was recorded by time-lapse photography, with one picture being taken every 2 sec for 9 hr daily. Infra-red photography was used during the night. Twenty-four different behavioural categories were analysed for each animal with mechanical and electrical aids. In addition, movement of each animal was automatically recorded; and (2) In monkeys, drugs were injected directly into the brain through permanently implanted cannulas; by means of an electrochemical micropump, activated by radio control. In other experiments, the drugs were given with the food.

Chlorpromazine (2 mg/kg, orally) increased balling (sleeping alone), did not affect nestling (sleeping in groups) and almost abolished active grooming. Reserpine (0·75 mg/kg, i.p.) was similar to chlorpromazine, but increased nestling and produced unusual patterns of mounting. Iproniazid (50 mg/kg, orally) increased several activities such as grooming, but also increased resting in groups. Pentobarbital (0·15 mg/kg, i.m.) abolished nestling and increased the amount of rest obtained without

social contact. Acetylcholine injected in the motor areas produced, after a short delay, motor effects in face and extremities. Some movements were discrete, and lasted for a few minutes; other responses, such as twitching, were more generalized and long-lasting. Acetylcholine injected in the hypothalamus produced electrical changes and only mild behavioural modifications. No response was observed when acetylcholine was injected in globus pallidus. The effects of other drugs on different anatomical structures will be shown.

441a Changements Induits par Drogues des Réflexes Conditionnés. J. MALMEJAC (Algérie).

Les réflexes conditionnés ont été utilisés sous deux formes complémentaires, à des fins d'études pharmacodynamiques:
(1) Réflexe salivaire (après fistulation du canal de Sténon) adapté aux conditions expérimentales. Pour s'assurer d'une "intégration" corticale, le chien est dressé, non avec un son pur, mais à l'aide d'une association de tonalités, d'une "mélodie pour chien". Par ailleurs pour pouvoir faire intervenir à différentes reprises le réflexe inné au cours d'une même séance afin de contrôler l'activité sous-corticale, celui-ci est déclenché par apport d'une cc de solution sapide sur la gueule de l'animal.
(2) Singes (*Macacus innus*) dressés à passer des tests avec reconnaissance de figures géométriques ou de couleurs ou à exécuter un "problem-box".

L'expérience réalisée montre que les modifications des réflexes conditionnés interviennent plus précocement que celles électroencéphalographiques, souvent difficiles à enregistrer sur un animal (surtout le singe) à l'état de veille.

La chlorpromazine, au-dessous du seuil d'action sur l'équilibre cardio-vasculaire, réduit considérablement le réflexe salivaire. L'alcool à faible dose (alcoolémies de 0·40 à 0·60 p. 1000) augmente les réflexes conditionnés (cette hyperexcitabilité corticale a été vérifiée secondairement chez l'homme). Les amphetamines augmentent les réflexes conditionnés. Une faible anoxie correspondant à une altitude de 3000 m, augmente les réflexes alors qu'une forte anoxie les réduit ou les supprime. L'adrénaline à aodse physiologique (1 à 2 μg/Kg/min) augmente l'excitabilité corticale. Un pentothal starter ou une anesthésie à l'éther ne troublent que passagèrement les réflexes conditionnés.

441b Drug Induced Changes in Conditioned Reflexes. J. MALMEJAC (Algeria).

Conditioned reflexes have been used in two complementary forms for the purpose of pharmacodynamic research:
(1) Salivary reflex (after fistulation of Stenon's duct) adapted to experimental conditions. To ensure cortical "integration", the dog was trained not with a pure sound but with a combination of tonalities—"a melody for the dog". To bring into action at different times during one session the innate reflex in order to control subcortical activity, this was released by savoury solutions applied to the mouth of the dog.
(2) Monkeys (*Macacus innus*) trained to pass tests with recognition of geometrical figures or colours or to execute a "problem box".

The experiments showed that the changes in conditioned reflexes precede those in the electroencephalogram, which are often difficult to record in animals (particularly in the monkey) in the alert state.

Chlorpromazine, below its threshold of action on cardio-vascular equilibrium, considerably lowered the salivary reflex. Low doses of alcohol (blood levels of 0·40–0·60 per 1000) enhanced the conditioned reflexes (this cortical hyperexcitability was subsequently checked in man). The amphetamines enhanced conditioned reflexes. Slight anoxia, corresponding to a height of 3000 m, increased the reflexes, while strong anoxia reduced or suppressed them. Adrenaline in physiological doses (1–2 μg/kg/min) increased cortical excitability. A pentothal starter dose or ether anaesthesia produced only transitory disturbances in the conditioned reflexes.

442 Glycogen and Phosphorylase Distribution Throughout the Walls of the Heart and Conduction System. L. A. JEDEIKIN (U.S.A.).

The distribution of glycogen across the walls of the heart has been studied in three species: rabbit, dog and ox, and the distribution of phosphorylase has been studied in rabbit hearts. Cardiac arrest was producing by plunging the excised heart into ice water. The right and left ventricles and septum were removed as quickly as possible and sectioned from endocardium to epicardium. In each species there was a marked and similar diminution of glycogen concentration from endocardium to epicardium, a two to five-fold difference being observed. Phosphorylase concentration in the rabbit heart varied in the same direction as for glycogen, but the gradient was much smaller, with an average drop of about 40 per cent from endocardium to epicardium. In the thick-walled ventricles of the dog and ox, the high concentration of glycogen in the endocardium fell abruptly and glycogen values continued at the lowest concentrations to the epicardium. In the septum the concentrations of glycogen were low in the centre of the wall, with a steep rise occurring at both surfaces.

The ox heart was of particular interest, because it was possible to dissect out the A-V node and bundle of His. The bundle of His had the highest concentration of glycogen found—at least three times greater than that of any other part of the myocardium. The studies on the beef heart indicate that the higher glycogen values are associated with the distribution of the conduction system, and the lower values may represent truer myocarial values.

443 The influence of the Autonomic Nervous System on the Activity of Cardiac Phosphorylase. M. E. Hess, J. Shanfeld and N. Haugaard (U.S.A.).

The cardiac phosphorylase in decapitated rats is extremely high. Activity of this enzyme can be decreased by modifying the sympathetic stimulation accompanying decapitation. In order to obtain more precise autonomic stimulation an open-chest rat preparation was used; the carotid artery was cannulated for measurement of blood pressure, drug injections were made through the external jugular vein, and isometric force of contraction of the heart was determined by means of a Walton–Boniface strain gauge attached to the apex of the heart and recorded through a Sanborn amplifier and oscillograph. The sympathetic system was stimulated by injecting dimethylphenylpiperazinium (50 μg/kg) into an atropinized animal. Following injection of this drug there was an increase in blood pressure and force of contraction and an increase in active phosphorylase in the heart. These effects were potentiated by prior injection of bretylium tosylate (5 mg/kg). Parasympathetic stimulation was elicited by electrical stimulation of the vagus or by injection of acetylcholine (50 μg/kg). Both of these procedures which reduced the blood pressure and cardiac rate, also lowered the phosphorylase activity of the heart.

Other investigators have shown that certain drugs which stimulate the mechanical activity of the heart, do not increase phosphorylase activity. Similarly, cardiac depressant agents generally do not decrease enzyme activity. It would appear, therefore, that the autonomic nervous system plays a unique role in governing the level of phosphorylase activity in the heart.

444 Dissociation of the Augmentation of Cardiac Contractile Force from the Activation of Myocardial Phosphorylase by Catecholamines. S. Mayer, M. deV. Cotten and N. C. Moran (U.S.A.).

Recent work from Haugaard's laboratory[1] and from our group[2] has indicated that there is a close relationship between the activation of phosphorylase and the positive inotropic action of catecholamines. This suggested that the enzyme activation is analogous to the interaction between the amine and the muscle contraction-producing receptor, or that perhaps augmentation of the enzyme is essential for the positive inotropic effect. The following experiments cast doubt on the relationship: (1) Doses of epinephrine of less than 0·5 μg/kg given intravenously to anaesthetized dogs produce significant increases in contractile force but not in phosphorylase activity. (2) The continuous infusion of large doses (10 μg/kg/min epinephrine) results in maintained increased contractile force while phosphorylase rises and then tends to return to normal. (3) In dogs where body temperature is reduced to 29–30°C the administration of ouabain (0·075 mg/kg) converts the effect of a subsequent injection of catecholamines from a positive to a negative inotropic one, but this effect is still accompanied by an increase in active phosphorylase. Dichloroisoproterenol (5 mg/kg, i.v.) blocks the negative inotropic effect and also the enzyme augmentation. Thus where catecholamines increase contraction of cardiac muscle, phosphorylase activation does not necessarily occur, and where the effect is to decrease contraction, enzyme activation is still observed.

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1. Kukovetz *et al.* (1959), *J. Pharmacol.*, **127,** 122.
2. Mayer and Moran (1960), *J. Pharmacol.*, **129,** 271.

445 Inhibitory Effect of Acetylcholine on Glycogenolysis in the Heart. S. Ellis and N. H. Vincent (U.S.A.).

In isolated perfused guinea-pig hearts, acetylcholine inhibited the glycogenolytic effect as well as the chronotropic and inotropic effects of epinephrine. When the heart rate was controlled by electrical stimulation, acetylcholine, without modifying the rate, inhibited epinephrine glycogenolysis. Atropine antagonized all the inhibitory actions of acetylcholine. Epinephrine produced positive inotropic and glycogenolytic effects, but no chronotropic effect, in the presence of veratramine. Dichloroisoproterenol inhibited all three effects of epinephrine. Acetylcholine also inhibited glycogenolysis induced by theophylline. Acetylcholine produced a partial, but significant, inhibition of anoxic glycogenolysis in the atria, but not in the ventricles. On the basis of the above results a metabolic role for acetylcholine in the control of cardiac glycogen catabolism is proposed. These findings also suggest a possible biochemical basis underlying the physiological control of cardiac function by acetylcholine.

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446 The Influence of Pharmacological Agents on the Coronary Chemoreflexes. V. V. Zakusov (U.S.S.R.).

Experimental studies on the influence of analgesic and neuroplegical drugs on coronary chemoreflexes were undertaken at the author's laboratory in view of their paramount important in the development of many pathological conditions.

Coronary chemoreflexes (bradycardia and hypotension) were elicited in anaesthetized cats by infecting serotonin or veratrine into the left coronary artery or into the cavity of the left ventricle.

Analgesics (morphine, promedol, phenadoxone) are capable of depressing the coronary chemoreflexes to a considerable extent. Among neuro-

plegic drugs, aminazine will likewise depress these reflexes, while reserpine suppresses serotonin-induced reflex bradycardia, but exerts no definite influence on cardiac deceleration caused by veratrine.

As substances eliciting coronary chemoreflexes may form in the heart itself when the coronary circulation is disturbed, it was decided to test the influence of analgesic and neuroplegic agents on the reflex changes of circulation brought about by limiting the coronary blood-flow. It was found that the above agents will decrease the reflex response to clamping of the coronary artery.

Analgesic and neuroplegic drugs will likewise decrease the reflex contraction of the coronary vessels when the afferent nerves are stimulated.

The coronary chemoreflexes have much in common with the reflexes in response to chemical stimulation of the lungs.

447 The Influence of Pharmacological Agents on the Reflex Reactions of Coronary Vessels. N. V. Kaverina (U.S.S.R.).

One of the ways of normalizing the cardiac blood supply consists in suppressing reflex reactions causing contraction of the coronaries with the aid of pharmacological agents.

The aim of the present investigation was to study the influence of pharmacological agents on the reflex reactions of the coronaries following stimulation of the receptors of the carotid sinus and of the afferent nerves.

As has been shown earlier by us, certain compounds of the phenothiazine series (chloracyzine, mepazine and to a lesser extent aminazine) increase the cardiac blood supply. It seemed interesting to elucidate the role played of these agents in the reflex reactions of the coronaries tending to return the blood supply the myocardium to normal. It was found that aminazine and mepazine cause considerable depression of the reflex reactions of the coronaries. Chloracyzine's influence on these reactions is inconstant. Apparently, the normalizing effect on the coronary circulation exerted by the phenothiazine compounds studied is due to different mechanisms.

Analgesic agents (morphine, thecodin, promedol, phenadon) in doses corresponding to those used therapeutically, depress the reflex reactions of the cardiac vessels. Nitroglycerine depresses considerably the reflexes reactions, though it does not alter materially either the minute volume of the coronary blood flow or the resistance of the cardiac vessels. This effect is due to the influence it exerts on the central nervous system.

Thus, the property of pharmacological agents to alter the intensity of the coronary reflexes may be an important link in the mechanism of their influence on the cardiac blood supply.

448 The Action of Drugs on Circulatory Disturbances in the Rabbit Heart due to Anaphylactic Conditions. E. Busch (Germany).

Exploration of coronary vasodilators is complicated by the fact that experimental conditions are required which imitate as far as possible pathological conditions in man. In the protein-sensitized rabbit, conditions are very similar to human coronary circulatory disturbances. Apart from myocardial changes, the coronary vessels are damaged severely which results in circulatory disturbances in the myocardium, changes in the electrocardiogram, and hypotension if a shock is induced. These symptoms can be observed during an anginal attack or during the development of an infarct. 1-*p*-ethoxyphenyl-3-diethylamino-indan which has been developed in our laboratories is a coronary active substance and compensates consequences of a shock or, if given before the shock, will prevent symptoms. Comparative trials with vasopressin or ergometrin, used until now to induce a spasm, have shown that this drug causes myocardial disturbances, but does not alter general circulation correspondingly. (It must be mentioned here that vasopressin and ergometrin-induced spasms can also be compensated by the above new substance.) The difference between the two forms of circulatory disturbances may be explained by the fact that vasopressin and ergometrin spasms affect a healthy organ which has sufficient reserves to tolerate this stress for a certain time. However, functional coronary constriction without pathological changes of vessels and heart muscle—so-called angina pectoris vasomotorica—is rather controversial, therefore, these tests are only of limited value when the coronary activity of a substance is to be judged. Today an anaphylactic process as the cause of human coronary disturbances is frequently discussed. Hence, we are of the opinion that an anaphylactic myocardial ischemia in the rabbit is particularly suitable to study the effectiveness of coronary vasodilatators.

449a Mode d'Action des Inhibiteurs de la Mono-Amine-Oxydase sur le Systeme Cardio-Vasculaire—Relations avec la Structure Chimique. J. Cahn, M. Herold, N. Barre, O. Kabacoff and I. Bamberger (France).

Les auteurs ont étudié les effets sur le système cardio-vasculaire de différents inhibiteurs de la mono-amine-oxydase (Harmaline, Marsilid, Tersavid, Marplan, RO 4 1038, RO 4 2637, Niamide, PC 603, PC 607, PC 610, PC 614); les effets pharmacologiques de ces inhibiteurs de la mono-amine-oxydase sur le système cardio-vasculaire et l'étude de la dynamique et du métabolisme cardique ont été étudiés chez 60 chiens; les effets de ces mêmes drogues sur les modifications E.C.G. provoquées par la vasopressine ont été recherchés sur 100 lapins; l'action de ces mêmes drogues sur la nécrose

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cardiaque provoquée par l'*iso*propyl-artérénol a été déterminée sur 100 rats, à la fois du point de vue des modifications histologiques et des modifications de l'E.C.G.

Dans la plus grande majorité des cas les effets constatés sur ces différents paramètres ne sont pas proportionnels à l'intensité d'inhibition de la mono-amine-oxydase *in vitro* ou *in vivo*; ceci est extrêmement net en ce qui concerne le débit coronaire et la consommation en oxygène du ventricule gauche. Les effets de ces inhibiteurs sur la pression artérielle sont dus aux modifications du débit cardiaque et de l'efficacité mécanique du ventricule gauche. L'action la plus nette des inhibiteurs de la mono-amine-oxydase est d'économiser la consommation en oxygène du myocarde; ceci paraît être dû à une stimulation vagale cardiaque et ressemble aux effets de la Sérotonine.

Les auteurs discutent à ce propos de la théorie de Raab et insistent sur les effets particuliers d'inhibiteurs de la mono-amine-oxydase dérivés d'hormones de croissance (PC 603-607-610) ou d'acides gras en C_4 (PC 614) par opposition au Marsilid et à ses dérivés.

449b Mode of Action of Inhibitors of Mono-Amine-Oxydase on the Cardio-Vascular System–Relationship to Chemical Structure. J. Cahn, M. Herold, N. Barre, O. Kadacoff and I. Bamberger (France).

The authors have studied the effects of various inhibitors of mono-amine-oxydase (Harmaline, Marsilid, Tersavid, Marplan, RO 4 1038, RO 4 2637, Niamide, PC 603, PC 607, PC 610, PC 614) on the cardio-vascular system; the pharmacological effects of these inhibitors of mono-amine-oxydase on the cardio-vascular system and changes in cardiac metabolism were studied on 60 dogs. The effects of these same drugs on modifications of the ECG, caused by vasopressin, have been studied on 100 rabbits. The action of these drugs on cardiac necrosis, provoked by *iso*propyl-arterenol was determined on 100 rats by means of histological investigations and ECG records.

In the great majority of cases, the effects which were observed on these different levels were not proportional to the degree of *in vitro* or *in vivo* inhibition of mono-amine-oxydase. This is very clear in regard to coronary insufficiency and oxygen consumption by the left ventricle. The effects of these inhibitors on arterial pressure are due to modifications of coronary insufficiency and of the mechanical efficiency of the left ventricle. The most striking effect of the inhibitors of mono-amine-oxydase is to economize on oxygen consumption by the myocardium; this appears to be due to vague stimulation of the heart and resembles the effects of serotonin.

The authors discuss Raab's theory and stress the special effects of the mono-amine-oxydase inhibitors, derived from growth hormones (PC-603-607-610) or fatty acids with C_4 (PC 614) in contrast with those of Marsilid and its derivatives.

450 Pharmacological Analysis of Neurogenic Myocardial Lesions. Z. I. Vedeneyeva (U.S.S.R.).

The fact that adrenaline and noradrenaline administered in massive doses to an animal may cause myocarditis, or rather myocardial generation, is well known. The question arises, whether these same lesions may not develop under the action of adrenaline- and noradrenaline-mediators excreted at the terminals of the sympathetic nerve in the heart after excessive irritation of the sympathetic nerve.

Accordingly, experiments were undertaken upon albino rats. In one series, the animals received a single dose of adrenaline or noradrenaline, in another, under light ether anaesthesia, one of their ganglia stellata was laid bare and traumatized by means of haemostatic pincers.

It was shown that excessive irritation of a ganglion stellatum caused the same kind of lesion in the myocardium as that observed after massive doses of adrenaline and noradrenaline, but less intense and developing more slowly. A pharmacological analysis of the phenomenon induces us to think that the destructive action of adrenaline is due to its sympathomimetic activity upon adrenoreactive systems of the myocardium. The results obtained, together with the data gathered from previously published works, lead to the conclusion that sympathetic nerves, which in normal conditions regulate the trophic processes of the myocardium, may, under the action of extraordinary impulses, impair these same processes, and cause dystrophic and destructive lesions. Most probably, such impulses are transmitted through the fibres of the cardial sympathetic nerves.

451 Cardiovascular Responses following Injection of Picrotoxin into the Lateral Cerebral Ventricle of Rabbits. D. R. Varma, N. N. Share and K. I. Melville (Canada).

Blood pressure and electrocardiographic (Leads II & V) changes of anaesthetized (pentobarbitone), vagotomized and curarized (gallamine) rabbits on artificial respiration, were recorded before and after injection of 0·1 mg picrotoxin into the lateral cerebral ventricle. Within 3–10 min after picrotoxin, marked cardiac arrhythmias (ventricular extrasystoles, paroxsymal auricular or ventricular tachycardia, and even ventricular fibrillation) ensued, followed (in the absence of fatal ventricular fibrillation) by regular rhythm and pronounced S-T depression (2–8 mm) lasting 30–120 min. Blood pressure increase (40–80 mm Hg) appeared 30 sec–3 min after injection and lasted 30–60 min. Glyceryltrinitrate antagonized the S-T depression to varying degrees (6 exps.). Pretreatment with

reserpine or phenoxybenzamine (3 exps. each) abolished the cardiovascular responses to picrotoxin. After LSD-25 (20 min earlier) picrotoxin-induced ventricular fibrillation within 5 min in all 3 exps. After iproniazid (1 hr earlier) arrhythmias and S-T depression ensued in all 3 exps., although ventricular fibrillation was not observed. Spinal section (T4–5) or bilateral adrenalectomy (2 exps. each) did not abolish the responses. It is concluded that picrotoxin stimulates central sympathetic mechanisms leading to increased sympathetic outflow. These findings suggest that central nervous system controlling mechanisms might be important in initiating both myocardial ischemic changes as well as cardiac arrhythmias. This procedure also appears to offer promise as an experimental method for studying these latter types of changes.

(Supported by a Provincial Health Grant and a grant from the Quebec Heart Foundation.)

452 The Influence of Pharmacological Agents on the Evolution of Experimental Myocardial Infarction of the Rabbit. A. A. Miazdrikova (U.S.S.R.).

The present paper reports the study of influences exerted by different drugs on the development of the collaterals and on the evolution of myocardial infarcation in rabbits. Vaso-dilators, possessing different mechanisms of action, were tested (nitroglycerine, papaverine, chloracysine, Hexonium, pentamine and mecamine).

Three to four days after the ligation of the left coronary artery all these drugs (except nitroglycerine) were injected intramuscularly as a 1 per cent solution, in doses of 3 mg/kg of body wt., daily for 30 days. In the case of nitroglycerine 0·1 ml was placed on the oral mucosa. The condition of the coronary circulation was judged by changes of blood pressure, electrocardiogram and by development of collaretals in the myocardium. The last was done with the help of roentgenography.

Data obtained show that chloracyzine, papaverine and pentamine speed up normalization of changes in the ECG and blood pressure and accelerate the development of collaterals in the myocardium. The effect of hexonium and mecamine was less favourable. Nitroglycerine failed to produce any significant changes in the evolution of experimental infarction as compared to the control group of animals.

453 The Effect of Some Vasoactive Drugs on the Blood Flow in Experimentally Produced Myocardial Infarction and on the Cardiac Output of the Infarcted Heart. G. Lange (Germany).

In open-chest dogs the blood flow of the heart muscle was measured simultaneously in an infarcted and in a non-infarcted area with the calorimetric method of Kiese and Lange.[1] An isotopic dilution method according to the principle of Stewart and Hamilton[2] was used for the determination of the cardiac output. In the infarcted areas the blood flow was diminished by 50 per cent or more. The infarction was followed by a decrease in blood pressure and cardiac output, frequently after a short increase of the latter. Vasodilator agents, as aminophylline, increased the blood flow in infarcted as well as in non-infarcted areas and increased the cardiac output as long as no considerable drop in the blood pressure occurred. The following acted similarly: papaverine, nitroglycerin, 2:6-bis (diethanolamino)-4:8 - dipiperidino - pyrimido(5:4 - d)pyrimidine, 2 - ethyl - 3(3′:5′ - diiodo - 4′ -hydroxy - benzoyl) - benzofurane, N - 3′ - phenyl - propyl - (2′) - 1:1 - diphenyl-propyl-(3)-amine- tolazoline, adenine nucleotides, rauwolfia alkaloids, khelline, chlorpromazine, tetraethylammoniumbromide, azapetine, atropine. By increasing rates of infusions, a dosage for all drugs could be found which augmented the coronary blood flow without an extensive fall in blood pressure. In doses which increased the blood pressure, norepinephrine augmented the coronary blood flow more than epinephrine and was almost as effective as epinephrine in increasing cardiac output. Hypertension and vasopressin which in large doses decreased the blood flow of the infarcted and non-infarcted muscle and the cardiac output increased either in small doses. None of the drugs affected the blood flow in the infarcted area without changing the blood flow of the rest of the myocardium in the same direction.

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1. Kiese and Lange (1957), *Arch. Exp. Path. Pharm.*, **231,** 149.
2. Frimmer, Lange and Resag (1961), *Arch. Exp. Path. Pharm.* In press.

454 Synthetic Oxytocin (Syntocinon) As An Antagonist of Cardiac Ischaemic Changes. K. I. Melville and D. R. Varma (Canada).

Myocardial ischaemic changes (ST-T deviations in ECG) were induced: (a) by inhalation of 10 per cent oxygen in nitrogen in normal and atherosclerotic rabbits (maintained on 6 per cent corn oil and 2 per cent cholesterol for 4 to 9 months); (b) by intravenous injection of vasopressin (1 I.U./kg) in normal rabbits under pentobarbital anaesthesia; and (c) by intraventricular (right lateral cerebral ventricle) injection of picrotoxin (0·1 mg) in normal rabbits under light pentobarbital anaesthesia with the vagi cut, curarized and on artificial respiration. Oxytocin (1–5 I.U./kg I.V.) was found temporarily to reverse the ST-T changes induced by hypoxaemia in normal and atherosclerotic rabbits (10 exps.), to reduce or abolish the depressor responses and the ST-T changes induced by vasopressin (8 exps.) and to reduce the ST-T changes induced by intraventricular picrotoxin

(5 exps.). Oxytocin also reversed ventricular fibrillation and restored blood pressure in 6 out of 9 rabbits, following fibrillation induced by intraventricular picrotoxin; spontaneous ventricular fibrillation recorded in two reserpinized cats on artificial respiration was also restored to sinus rhythm. Chloroform–adrenaline fibrillation induced in 2 dogs was interrupted without blood pressure restoration. These preliminary observations strongly suggest that oxytocin is an effective antagonist of myocardial ischaemia produced by different methods, and may prove to be useful in angina pectoris and as an antifibrillatory agent. This antagonism may also be an important physiological function of the oxytocic hormone, possibly related to the lower incidence of coronary ischaemia attacks in women.

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455 The Nature of Quinidine-Protein Binding with Reference to Myocardial Effects of Quinidine. H. L. Conn, Jr., R. J. Luchi and J. Helwig (U.S.A.).

The basic reactions responsible for the electromechanical disturbances produced in the myocardium by quinidine are not known. From studies of quinidine's influence on sodium and potassium transfer and distribution in the heart, a prime action of the drug on membrane permeability was suspected. Since the effect might depend on the nature of drug binding to protein and to membrane material, these matters were studied in an *in vitro* dialysis system. Quinidine binding to albumin proved to involve interactions of the basic quinoline ring, quinoline nitrogen, methoxy group, and the alcoholic OH bridge with corresponding albumin sites, probably through formation of hydrogen bonds having a free energy of about 5 kcal. The charged quinuclidine nitrogen remains free. There is only one receptor area for quinidine in the albumin molecule at physiological pH's. H^+, Cl^-, and Ca^{++} partially inhibit the reaction. Preliminary studies indicate a similar reaction between quinidine and *E. coli* bacterial membrane and red cell membrane, although the association constants may be greater. The hypothesis was made that quinidine has a detergent-like surface action, the quinoline fragment being bound to surface protein and the quinuclidine N^+ reacting with aqueous polar groups. At a membrane surface these oriented charges could alter membrane permeability to ion movement and secondarily electrical and possibly mechanical activity. Evidence compatible with this thesis was obtained in dogs by the intravenous administration of 4-hydroxy-methyl-6 methoxyquinoline, quinidine without the quinuclidine ring. In accordance with our view, this substituted quinoline with the N^+ proved to have no myocardial electro-mechanical depressant properties.

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456 Urinary Excretion of Drugs. L. Peters (U.S.A.).

The renal clearance of different drugs may range from rates far below glomerular filtration rate (GFR), to high rates consistent with virtually complete extraction in one renal circulation. Low clearance rates may be based on binding of drugs to plasma proteins, or passive back diffusion of the non-ionic lipid-soluble form of the drug from tubular lumen to peritubular capillaries. Some drugs are also excreted by the renal tubules via active transport systems, presumably intended for excretion of metabolites, but capable of excreting drugs with certain chemical configurations. Two such systems have been characterized. One excretes organic acids such as phenol red, hippurates, Diodrast and the penicillins. The other excretes organic bases such as histamine, guanidine and tolazoline, and numerous quaternary ammonium compounds. Transportable acids compete for tubular excretion as do transportable bases, but competition between the two groups is not demonstrable. Maximal tubular transport capacities have been demonstrated for some compounds in each group. Renal clearance rates of some organic bases (e.g. mecamylamine) vary inversely, and of some organic acids (e.g. salicylic) directly, with urine pH. Thus, the clearance of mecamylamine is below GFR when the urine is alkaline but far exceeds GFR when the urine is strongly acid. Various investigations suggest that this excretory pattern involves a three-phase system, namely filtration at the glomerulus, active tubular excretion of the ionic form, high in the tubular lumen, and passive back diffusion of the lipid soluble non-ionic form, lower in the tubule where the urine is concentrated. Details of these excretory mechanisms will be discussed.

457 Duration of Action of Drugs as Affected by Tissue Distribution. T. C. Butler (U.S.A.).

Treatments of the kinetics of drug distribution based on pluricompartmental models of the body with intercompartmental exchange subject to various modes of constraint have been proposed by several authors. If the rate of passage of drug out of each compartment is assumed to be proportional to the concentration in that compartment, the concentration in any compartment as a function of time is expressed as a series of exponential terms. For many circumstances, this model approaches biological validity. Being analogous to a simple electrical network of capacitance and resistance, it lends itself to electrical analogue computations. Satisfactory conformity to experimental data can be achieved with mathematical functions generated by greatly simplified models. For many purposes a 3-compartment model suffices: one representing plasma, one some tissue of particular interest, and a third with properties composite of the remainder of the body. Simple 3- and 4-compartmental models

of the body and their analogous electrical circuits will be used to illustrate several phenomena in which distribution of a drug within the body determines duration of action.

Topics to be discussed include: blood flow and diffusion barriers as factors limiting interchange between plasma and tissues, prolonged duration of action due to slow absorption and due to interaction with constituents of a compartment, redistribution during recovery from intravenous and inhalation anaesthesia, intercompartmental shifts occasioned by changes of pH.

458 Discussion of the Previous Paper. E. Westermann (Germany).

459 Drugs as Inhibitors of Drug Metabolism. K. J. Netter (Germany).

This contribution will be concerned with drugs inhibiting the microsomal enzyme system in the liver that catalyses the metabolism of other foreign compounds. The system is capable of mediating a remarkably wide variety of different inactivation reactions. For maximum activity TPNH and oxygen are required. During recent years a number of organic substances of different character has been found to inhibit the metabolism of many drugs. An account will be given of the chemical nature of these compounds as well as of the reactions they inhibit. The group of the so-called microsome inhibitors comprises substances possessing well-defined activities apart from the inhibition of drug metabolism together with pharmacologically relatively inactive compounds. Among them the diethylaminoethanol esters of substituted acetic acid (SKF 525-A, CFT 1201) have been studied most extensively. Nevertheless, the exact mechanism of the inhibition remains unexplained. Solubilization or purification of the enzymes involved has not yet been achieved and might even be impossible because they might be bound tightly to the structure. Because of the chemical inhomogeneity of the active inhibitors, structure–activity considerations did not reveal a specific configuration to be necessary for inhibition. They led to the conclusion, however, that within the microsomes probably different enzyme systems are responsible for different types of reactions. Kinetic studies have been the only other approach to the problem so far. Most of them indicate a non-competitive inhibition. Also physical factors such as penetration rates may be affected by the inhibitors.

460 Discussion of the Previous Paper. B. LaDue (U.S.A.).

461 Drugs as Activators of Drug Enzymes. H. Remmer (Germany).

Pretreatment of rats with many different drugs stimulates the activity of enzymes in liver microsomes which oxidatively metabolize a variety of drugs. The activating nature of a substance has no relation to its pharmacological effect or to its chemical configuration. Solubility properties of the compounds play an important part. Strong activators are hypnotics, e.g. barbiturates, ureides and hydantoins, but ethyl alcohol, ethinamate, the unsubstituted barbituric acid, meperidin and morphine do not have any activating effect. Drugs stimulating the CNS, e.g. bemigride and nikethamide, but not metrazol, are strong activators. Aminopyrin, phenylbutazone and orphenadrine have the same effect. A very strong activator is the antidiabetic substance tolbutamide, but not the closely related carbutamide.

These drugs exert the strongest stimulation on the oxidizing enzymes, but other enzymes in the liver microsomes, e.g. the procainesterase can probably be activated too. An interesting, still poorly understood correlation exists between the activation of these enzymes and the stimulation of the synthesis of glucuronic acid and ascorbic acid.

Besides drugs which activate microsomal enzymes other substances are known which have an opposite effect and inhibit these enzymes, but inhibition and activation are closely related.

Activating substances can inhibit the oxidizing enzymes and inhibiting compounds can also activate under certain conditions.

A stimulation of the hypophyseal-adrenocortical system does not play any part in the activation of enzymes. Some indication exists that the activation may be an adaptation with an increase of enzymes. But it was not possible to detect any change in the protein, lipoid and nucleic acid content of the microsomes with activated enzymes.

The activation of drug metabolizing enzymes is important in therapy. The tolerance to certain barbiturates depends on an accelerated oxidation. The oxidative demethylation of aminopyrin increased in patients for some weeks after intoxication with barbiturates and related hypnotics.

462 Discussion of the Previous Paper. A. Conney (U.S.A.).

463 Physiological Impairment of Drug Metabolism. J. R. Fouts (U.S.A.).

We have been interested in physiological or pathological states which affect the rate of metabolism of drugs by hepatic microsomes. In choosing conditions likely to affect these enzyme systems, we were guided by electron micrographs of the structure of the endoplasmic reticulum. We have postulated that there is a relationship between the structure of the endoplasmic reticulum and the activity of the enzyme systems localized in the microsomal fraction. Marked changes in the amount or organization of the vesicles of the endoplasmic reticulum have been associated with effects on the rate of metabolism of drugs by hepatic microsomes.

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Rapidly-growing hepatic cells have an unusual type of endoplasmic reticulum. We have shown that certain drug-metabolizing enzyme activities are depressed in microsomes from livers of newborn animals, livers regenerating after partial hepatectomy, and several types of hepatic neoplasm.

Obstructive jaundice and starvation lead to effects on reticulum structure and on some microsomal enzyme activities.

Finally, we have investigated recently the interrelationships of hepatic glycogen, reticulum structure, and drug-metabolizing enzyme activities. Severe depletion of glycogen leads to structural changes in the reticulum, and also affects microsomal enzyme activities. Livers from rats made diabetic with alloxan have little ability to metabolize various drugs. These effects of diabetes are reversed by treating the animal with insulin. We believe the effects of starvation on the microsomal enzymes may similarly be related to effects on hepatic glycogen.

464 Discussion of the Previous Paper. M. WEINER (U.S.A.).

465 Difficulties in Projecting Drug Metabolic Data from Animals to Man. J. J. BURNS (U.S.A.).

Marked species differences have been found in the metabolism of many clinically useful drugs such as phenylbutazone, meperidine, biscoumarin acetate, and pentobarbital. These metabolic differences make it difficult to extrapolate pharmacological data obtained in experimental animals to man. Another complication in the clinical evaluation of drugs is the marked individual variations found in their rate of metabolism. Administration of a dose of a drug to some human subjects may give plasma concentrations which are less than therapeutic, whereas in other patients the same dose may give plasma concentrations which are toxic. Individual differences in the metabolism of drugs may be due to genetic factors, and this has been suggested from finding differences in metabolism of drugs by inbred strains of certain animal species. Other factors will be discussed which may contribute to variations in drug metabolism. These include the simultaneous administration of other drugs, nutritional status, age, and sex. These metabolic variations may be explained in terms of the activity of drug-metabolizing enzymes in liver microsomes.

466 Biochemical Processes Involved in the Synthesis, Accumulation and Release of Triglycerides in the Liver. M. G. HORNING (U.S.A.).

A number of studies have shown that the administration of single large doses of ethanol and carbon tetrachloride to the rat cause a fatty liver and that the marked increase in liver lipids is due to an accumulation of triglycerides. Using the technique of gas chromatography, we have been able to show by analyses of liver and adipose triglycerides that the fatty acids of depot triglycerides are the source of the deposited liver triglyceride fatty acids. This accumulation of triglycerides after ethanol may be due to an increased mobilization of fatty acids from the depots since there is a marked rise in the plasma-free fatty acids. However, the increase in liver triglycerides after carbon tetrachloride administration is so great that it seemed likely that other factors were also involved. Therefore, the possibility of increased triglyceride synthesis, decreased oxidation of fatty acids, and inhibition of triglyceride release by the liver was considered.

The influence of ethanol and carbon tetrachloride on each of these processes has been studied. For example, *in vitro* experiments with liver homogenates from normal, and alcohol and carbon tetrachloride treated rats showed markedly different patterns of incorporation of [1-^{14}C] palmitic acid; the labelled palmitate present in the triglycerides from liver homogenates of ethanol and carbon tetrachloride treated rats was twice that of the control rats. Recovery of labelled acid from the control homogenates was consistently lower than from the homogenates of treated rats. Experiments designed to study release of triglycerides from the liver have also been carried out.

467 Drug-Induced Mobilization and Deposition of Triglycerides through Release of ACTH. R. PAOLETTI, R. MAICKEL and R. SMITH (U.S.A.).

Chlorpromazine and alcohol cause stimulation of the pituitary adrenal system as evidenced by rises in plasma corticosterone, liver tryptophane pyrrolase (TPO) and plasma-free fatty acids (FFA), in normal but not in hypophysectomized or adrenalectomized rats. These drugs also cause a marked increase in liver triglycerides (TGL) in normal but not in hypophysectomized rats.

Depot ACTH also elicits a rise in plasma corticosterone, FFA and in liver TPO and TGL in normal but not in adrenalectomized rats. The extent of these changes is almost identical with that observed after chlorpromazine and alcohol, indicating that the lipid changes induced by these drugs are mediated by ACTH hypersecretion. However, massive doses of depot ACTH caused some rise in FFA even in adrenalectomized rats.

Reserpine provokes an intense and sustained ACTH discharge and elicits the classical indices of adrenocortical stimulation but fails to increase liver TGL. Since reserpine depletes tissue catecholamines, a role of these amines in lipid mobilization by ACTH was suggested. In animals completely devoid of stored catecholamines (adrenal demedullated rats treated with reserpine) neither ACTH nor chlorpromazine caused an increase in plasma FFA and liver TGL. Additional evidence of the importance of peripheral catecholamines *in vivo* was

obtained using an adrenergic blocking agent (Dibenamine) and a long-lasting ganglionic blocking agent (Ecolid). These compounds completely blocked the lipid mobilizing effects of ACTH and alcohol.

The presence of measurable amounts of catecholamines has been demonstrated in the rat adipose tissue (0·15 μg/g). Possible interrelationship between noradrenaline, ACTH and lipid mobilization will be discussed.

468 Transport of Fatty Acids between Adipose Tissue and Liver: The Role of Catecholamines and the Sympathetic Nervous System. R. J. Havel (U.S.A.).

Injection of norepinephrine and epinephrine and factors promoting adrenal medullary secretion or sympathetic nervous activity, such as nicotine, psychic stress and upright posture, increase the rate of mobilization of free fatty acids (FFA) from adipose tissue into the circulation. Conversely, factors which decrease these functions, such as blockade of sympathetic ganglia and deep anaesthesia, decrease the rate of mobilization. Peripheral adrenergic blockade abolishes the mobilizing effect of injected catecholamines. The concept has been developed that the sympathetic nervous system has a tonic action promoting mobilization of FFA, which can be altered by central stimuli as well as by hormonal factors. The most notable of the latter is insulin, which promotes re-esterification of FFA in adipose tissue and thereby decreases their rate of mobilization.

Since FFA in blood plasma turn over very rapidly, their rate of mobilization can be expected to have profound effects upon lipid metabolism in other tissues. A large proportion of FFA are taken up by the liver, where they are rapidly esterified to form triglycerides. These are converted to other lipids or transported back into the circulation as constituents of very low density ($<1{\cdot}006$) lipoproteins. The rate of uptake in adipose tissue of fatty acids derived from these triglycerides depends upon several mechanisms involving nervous and hormonal interaction. Therefore, factors which influence metabolism of FFA in adipose tissue can affect both formation of plasma lipoproteins in liver and removal of their triglyceride constituents from the circulation. These factors thereby exert powerful control over lipoprotein triglyceride turnover and concentration in the blood.

469 Hormonal Control of Lipid Transport in the Isolated Perfused Rat Liver. M. Heimberg, I. Weinstein and H. Klausner (U.S.A.).

Livers were perfused through the portal vein, using a medium consisting of defibrinated rat blood diluted with a bicabonate buffer, pH 7·4, to three times its original volume. Bile flow, flow rate and bromsulphalein uptake were used as indices of liver function. The uptake of triglycerides from synthetic neutral fat emulsions or from tripalmitin labelled rat chylomicra was faster in livers from starved rats than from normal-fed animals. In the absence of added lipid to the perfusate, there was release of triglyceride from livers of fed animals, whereas there was a net uptake of the endogenous triglyceride by livers from fasting rats. Release of phospholipid paralleled that of triglyceride. Little change in perfusate cholesterol was observed. Isotopic evidence suggested that in fed liver, uptake and release of triglyceride occur simultaneously. In experiments using livers from castrate male rats, the release of triglyceride into the medium was greater than from normal animals. No difference in rate of uptake of endogenous serum nonesterified fatty acids or of added fatty acid–albumin complex was observed with either livers from fed or fasting animals. These results confirm previous experiments using buffer alone as perfusate.(1) In the latter system, uptake of triglycerides was proportional to concentration, and was increased by adrenalectomy and also by castration of liver donors. Hydrocortisone addition to the medium perfusing livers from adrenalectomized rats decreased triglyceride uptake to normal values in proportion to dose of steroid added. These results indicate an hormonal control of triglyceride transport in livers.

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470 The Actions of Adrenotropic Substances on Depot Lipids. M. Wenke, E. Mühlbachová and S. Hynie (Czechoslovakia).

The effects of α- and β-adrenomimetics on the lipolysis in depot fat tissues was estimated *in vivo* and *in vitro* (noradrenaline, isoproterenol, adrenaline). The interaction of these effects with the α-lytic phentolamine and the β-lytic dichlorisoproterenol have also been investigated.

In the *in vivo* experiments the content of unesterfied fatty acids (UFA) in the epididymal adipose tissue of rats was taken as the criterion for lipolysis. The level of UFA was increased almost equally by all adrenomimetics. This effect could be blocked by all adrenolytics used.

In vitro the release of UFA from adipose tissue into into the medium was determined when adding various amounts of adrenomimetics and adrenolytics.

Again, all mimetics used increased the lipolysis and it was impossible to find any quantitative differences between their effects. Both adrenolytics used blocked the effects of the adrenomimetic.

The results are discussed on the basis of Ahlquist's division of adrenomimetics which stimulate alpha receptors (effect: vasoconstriction, liver glycogenolysis) and beta receptors (effect: vasodilatation,

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bronchodilatation, tachycardia and muscle glycogenolysis). In these effects the alpha and beta qualities are clearly separated. In lipid metabolism, however, the situation is different. From the point of view of Ahlquist's theory this would mean that the lipolytic effect of adrenomimetic drugs is not caused only by alpha or only by beta receptor stimulation, nor by combined stimulation of both these receptors and so it would be necessary to presume a further, third type of receptor, which then would be gamma receptors.

471 Effect of Ethionine on the Metabolism of Chylomicrons in the Rat. B. Borgström, C. Naito and T. Olivecrona (Sweden).

Palmitic acid-1-^{14}C labelled chylomicrons has been injected into normal rats and rats with ethionine-induced fatty livers. The fatty liver rats remove the chylomicrons from the circulation at a much lower rate than the normals. The livers of the ethionine-injected rats show an appreciable accumulation of the chylomicron fat in the liver mostly due to a decreased outflow compared to normals.

472 Studies on the Mechanism of Fatty Liver Development in Ethionine-treated Rats. T. Olivecrona (Sweden).

Farber *et al.*[1, 2] showed that fasted female but not male or carbohydrate-fed female rats develop fatty liver when treated with ethionine. Using higher doses of ethionine we, however, get similar, although less massive, fatty livers in male and carbohydrate-fed female rats. Liver glycerides are severalfold increased whereas liver cholesterol and phospholipids are within normal ranges. Plasma of rats developing fatty livers show decrease of esterified fatty acids and slightly elevated FFA.

1-C-14-Palmitic acid bound to albumin after intravenous injection to normal rats is rapidly incorporated into esters and part of the injected label is recycled in plasma in esterified form. In functionally hepatectomized rats no such recycling takes place. In ethionine-treated rats, the liver takes up and esterifies the injected label at a normal rate. The activity in the liver, however, falls off at a slower rate than in normal rats. The plasma contains only small amounts of labelled esterified fatty acids.

The hypothesis is presented that under normal conditions the liver takes up FFA in excess of its demand, incorporates the excess into lipoproteins and redistributes the fatty acids to the peripheral tissues. In ethionine-treated rats lipoprotein synthesis is depressed and the resulting imbalance between uptake and release leads to the development of the fatty liver.

A tentative model for the transport of fatty acids in the rat is presented and discussed.

1. Farber, E., Simpson, M. V. and Traver, H. (1950), *J. Biol. Chem.*, **182,** 91.
2. Farber, E., Koch-Weser, D. and Popper, H. (1951), *Endocrinol.*, **48,** 205.

473a Action des Homologues de la Méthionine Étudiée par la Methode des Cultures in vitro. A. Cestari, C. Concilio, P. Dessi et C. Rizzoli (Italy).

Moyennant la substitution du méthyl de la méthionine par des chaînes aliphatiques ou aromatiques de poids moléculaire supérieur de C_2H_5, l'on obtient des thioéthers de l'homocystéine; certains de ces thio-éthers on perdu l'action stéatogène et toxique de l'éthionine tandis qu'ils conservent l'inhibition de la synthèse protéique au niveau cellulaire et subcellulaire (explants *in vitro* de coeur de poulet et de singe, schizomycètes, protozoaires) et dans les systèmes où l'accroissement est coordonné (embrions de poulet, rat albin).

Les dérivés: D, L, DL-n-propyl et *iso*propylhomocystéine; D, L, DL-n-butyl et *iso*butylhomocystéine; DL-octylhomocystéine; D, L, DL-benzylhomocyéine, additionnés au terrain sémi-synthétique de culture suivant Eagle, en concentrations croissantes de 0·025 à 0·5 pour cent, interfèrent profondément avec le métabolisme, l'accroissement et l'activité caryocinétique d'éléments épithéliaux humains carcinome HéLa).

L'analyse statistique a montré que l'inhibition de l'augmentation pondérale de l'explant diffère quantitativement, surtout en ce qui concerne les termes inférieurs de la série, suivant l'homologue présent dans le terrain de culture et, pour le même homologue l'inhibition dépend de l'activité optique.

Les considérations que l'on a pu tirer de la comparaison entre les différentes entités de l'inhibition provoquées par les deux antipodes optiques de chaque homologue, permettent un examen préliminaire des rapports qui courent entre la structure et l'activité des homologues de la méthiot ine.

473b A Study of the Action of Methionine by the Method of *in vitro* Cultures. A. Cestari, A. Concilio, P. Dessi and C. Rizzoli (Italy).

By means of the substitution of the methyl group of methionine by aliphatic or aromatic chains of molecular weights above C_2H_5, thioethers of homocysteine are obtained; some of these thioethers have lost the steatogenic and toxic properties of ethionin while retaining the property of inhibiting protein-synthesis at the cellular or subcellular level (*in vitro* explants of the chick or monkey heart, schizomycetes, protozoa) and in systems of organized growth (chick embryos, albino rats).

The derivatives: D, L, DL-n-propyl and *iso*propyl homocysteine; D, L, DL-n-butyl and *iso*butyl homocysteine; DL-octyl homocysteine; D, L, DL-benzyl-homocysteine when added to Eagle's semi-synthetic culture medium in concentrations rising from 0·025 to 0·5 per cent, interfere markedly with the metabolism, growth and karyocynetic activity of the epithelial elements in man (HeLa carcinoma).

Statistical analysis shows that the inhibition of the growth of the explant differs quantitatively, particularly with the lower values of the series, depending on the homologue present in the culture medium, and that inhibition by the same homologue depends on optical activity.

The conclusion which could be drawn from a comparison of the different degrees of inhibition caused by the two optical antipodes of each homologue, make it possible to explore the relationship between the structure and activity of the homologues of methionine.

474 The Influence of Some Pharmacological Agents on the Regulation of Lipid Metabolism. G. S. Gwishiani (U.S.S.R.).

These conclusions are confirmed by results obtained in irradiated rabbits whose heads were protected against irradiation.

The influence of some pharmacological drugs on the lipid content of the blood and of the aortic wall of rabbits with cholesterol induced atherosclerosis was studied.

The injection of novocaine into the animal organism is accompanied by a decrease in the cholesterol content of the blood and in the lipid composition of the aortic wall. The quantity of lecithin also decreases but to a slighter degree.

The intramuscular injection of diethylendoamino-cobalt iodide decreases the lipid content of the aortic wall. The quantity of cholesterol in the blood serum also decreases. The quantity of lecithin is not subjected to any definite changes.

The intramuscular injections of AKTG sharply decrease the cholesterol level in the blood and the lipid content of the aortic wall.

Some increase of the lecithin content of the blood serum is observed simultaneously.

475 The Influence of Chlorpropamide and Salicylate on Plasma Lipids. L. A. Carlson and J. Ostman (Sweden).

Salicylate, given intravenously to normal subjects as well as to patients with diabetes, decreases blood sugar. Simultaneously, a decrease of plasma-free fatty acids (FFA) is obtained. This decrease of FFA was studied with isotope technique and was found to be related to a diminished inflow to plasma of FFA. *In vitro* experiments have given evidence of a decreased output of FFA from adipose tissue after addition of salicylate, which agrees with the above-mentioned finding.

Chlorpropamide has an influence similar to salicylate on plasma FFA. Studies are in progress on the mechanism of this effect, which are hoped to be reported at the Meeting.

The long term effect of chlorpropamide on cholesterol, phospholipids and glycerides in plasma will be reported. The main effect observed, is a decrease of the glycerides.

476 Alterations in Serum Lipid Patterns and Fatty Acid Distribution after Oral Administration of Synthetically Prepared Arachidonic Acid. H. Wagener, H. Mai and G. Schettler (Germany).

Results of short- and long-term studies on the action of synthetically prepared arachidonic acid (administered as the ethyl ester) are presented. The study was undertaken with normals of different ages and persons with disturbed lipid metabolism, e.g. patients with atherosclerosis, essential hyperlipaemia, and essentially hypercholesterolaemia. The serums were analysed for total lipids, cholesterol, phospholipids, and total fatty acids. Total fatty acids were also separated by gas–liquid chromatography. Normal persons with different caloric intakes showed reduction of total lipids and total cholesterol within 24 hr with doses of 9, 3, and even 1 g ethyl arachidonate. The fall in total lipid and cholesterol levels appeared to be roughly proportional to the original levels. Hyperlipaemic and hypercholesterolaemic patients showed more marked depressions. The accompanying alterations in fatty acid distribution are presented and discussed.

477 Occurrence and Formation of Lysolecithin in Biological Material. E. Habermann (Germany).

Lipid components of tissues and body fluids can be separated by thin layer chromatography and can be determined subsequently. Quantitative data will be given, especially concerning the behaviour of the pharmacologically active lysolecithin under various conditions.

478 Reduction of 9α- and 12α-Halogenated 11-Ketosteroids. I. E. Bush and S. Hunter (United Kingdom).

Fried has demonstrated that the effect of halogen substituents adjacent to the 11-oxgen function of glucocorticoids is due to their negative inductive effect since fluorine has the same influence in increasing the activity of such steroids in position 9 and 12. It is believed that this effect increases the strength of hydrogen bonds with the 11β-hydroxyl group when this group acts as proton donor to the bond. Bush and Mahesh have shown that another

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effect is to prevent almost entirely the formation of 11-ketonic metabolites of 11β-hydroxy-steroids when they are administered orally to men. This is readily understood on thermodynamic grounds but needed checking with *in vitro* systems. Also it was predicted that large halogen substituents at position 12 (but not at 9) would inhibit the reduction of 11-ketone groups in such steroids.

We have been able to show that microsomes from rat livers reduce both 9α-fluoro- and 12α-fluoro-ketones much more rapidly than the unsubstituted analogues. This is a completely general effect and is much the same with the derivatives of 11-keto-progesterone, 11-dehydrocorticosterone, and cortisone. On the other hand, there is little or no reduction of the 12α-bromo derivatives of the former two compounds. These results confirm the earlier suppositions of Bush and Mahesh and correlate extremely well the biological activities of these steroids and their ability to be reduced at position 11. They also confirm the suggestion of these authors that 11β-hydroxy-steroids act on their tissue receptors as the 11β-hydroxy forms *per se* and that oxidation-reduction of the 11-oxygen function is not involved directly in the mode of action of such hormones and their analogues.

479 Relationship between Steroid Structure and the Renal β-Glucuronidase Response to Androgens. W. H. Fishman (U.S.A.).

Androgens, as a class, elicit an increase in the β-glucuronidase activity of mouse kidney.[1, 2] Corticosteroid hormones, in a few cases, had slight stimulating power whereas estrogens, thyroxine, and progesterone had none.[2] In the androgen series, it was established that the potent steroids were those which exhibited a preponderance of protein anabolic activity over virilizing action.[4] Amongst these steroids can now be included a number which lack the C-19 methyl group (19-nortestosterone, (17α-alkyl-19-nortestosterones, norandrost-5-en-olone phenyl propionate), some in which ring A is reduced and possesses a 2α-methyl group (androstane-3β-ol-17-one; 2α-methyl-dihydrotestosterone, 2α,17α-dimethyl-dihydrotestosterone) and others of diverse chemical structures (9α-fluoro-11β-hydroxy-17α-methyltestosterone, estrenol, "dianabol", 4-chlorotestosterone; 9α,11β-dichloro-16α methyl-$\Delta^{1,4}$-androstadien-17β-ol-3-one; "androstanazole". It is noted that a compound which enhanced β-glucuronidase in mouse kidney also increases the weight of rat levator ani muscle as a rule, although there are exceptions. The understanding of this phenomenon is aided by the recognition by enzymorphologic technique of the androgen-sensitive cells in the kidney and by the demonstration (Pettengill) that ^{14}C-labelled glycine is incorporated into the kidney β-glucuronidase protein. Finally, a picture is presented of possible metabolic events in Rings A and D of androgens[5] which merit consideration with regard to the interpretation.

1. Fishman, W. H. and Farmelant M. (1953), *Endocrinol.*, **52,** 536.
2. Riotton, G. and Fishman, W. H. (1953), *Endocrinol.*, **52,** 692.
3. Fishman, W. H., Artenstein, M. and Green S. 1955), *Endocrinol.*, **57,** 646.
4. Fishman, W. H. and Lipkind, J. S. (1958), *J. Bid. Chem.*, **232,** 729.
5. Fishman, W. H. (1961), *Chemistry of Drug Metabolism.* Charles C. Thomas, Publisher.

480 Chemical Structure as a Basis for Competitive Inhibition between Convulsants and Anticonvulsants. R. K. Richards (U.S.A.).

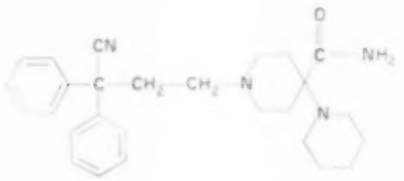
This laboratory has shown earlier that the convulsions caused by procaine in guinea pigs can be greatly reduced by prior injection with either of the two split products of procaine itself, namly, diethylaminoethanol or *p*-aminobenzoic acid. Compounds related to either diethylaminoethanol or *p*-aminobenzoic acid which protect against procaine convulsions follow definite structural patterns. The specificity of these anticonvulsants actions was demonstrated by the inability of these compound to protect animals against unrelated convulsants. Quaternization of procaine or its dimethylamino analogue results in compounds almost devoid of local anaesthetic action. If these quaternized derivatives are injected prior to the closely related tertiary one (for instance, the quaternary triethylamino procaine prior to procaine) a marked reduction of convulsions followed but not with the quaternized trimethyl homologue. For further investigation the following compounds were prepared: (1) the diethyl- and dimethylaminoethanol esters of diphenylacetic acid; and (2) their quaternized triethyl and trimethyl derivatives. Dose–response curves were constructed showing the protective action of each of the two quarternized compounds against the convulsant effect of each of the two tertiary compounds. These curves are compatible with a competitive inhibition. The quaternized compounds exert marked protective effect against their most closely related and to a much smaller degree to the less closely related tertiary compound. In some instances reversals of the protective effects occur with increasing doses of a quaternary compound, resulting in an increased incidence of convulsions. The question of penetration of the quaternized derivatives and the possibility that the competitive inhibition may occur on the blood–brain barrier rather than on central synapses is being investigated.

481 On the Unusual Chemical Structure of Piriniramide (R 3365), a Potent Analgesic. C. VAN DE WESTERINGH (Belgium).

The results of a systematic investigation on the qualitative and quantitative influence of modifications on 4 different points of the Pethidine molecule on the pharmacological properties are discussed shortly.

Most of the compounds have either analgesic or neuroleptic properties, the type of activity depending largely on the nature of the substituent on the nitrogen atom and to a lesser degree on the other modifications.

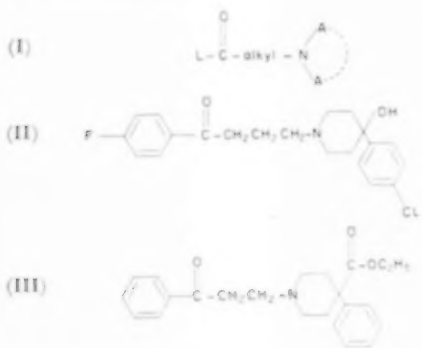
During this investigation it was found that substitution of the 4-phenyl group by other groups of basic, non-aromatic character (e.g. piperidine), in combination with other modifications, gives rise to compounds with novel properties. The most interesting of these compounds, Pirinitramide (R 3365; I) is a potent analgesic, about 5 times as active as pethidine. In dogs, however, R 3365 is devoid of morphine-like physical dependence capacity. The pharmacology of this compound is discussed in some more detail.



I

482 The Relationship between Chemical Structure and CNS-Depressant Activity of Basic Ketones Related to Haloperidol. P. JANSSEN (Belgium).

A series of over 1500 basic ketones of general structure I were synthetized and screened for CNS-depressant activity.



Many butyrophenones related to Haloperidol (II) were found to be potent neuroleptic drugs, pharmacologically similar to the phenothiazine derivatives of the chlorpromazine type, whereas many propiophenones related to compound R 951 (III) are potent morphine-like analgesics. The quality and the potency of the pharmacological effects produced by compounds of structure I depend on the nature of L, alkyl and NAA′. These relationships will be discussed in some detail.

483a Pharmacologie des Iodosynéphrines. R. MICHEL, J. KLEPPING, R. TRUCHOT et H. TRON-LOISEL (France).

L'iodation en 3 et 3:5 renforce et prolonge les principales propriétés sympathomimétiques de la Synéphrine (S). Chez le lapin, la 3-iodosynéphrine (MIS) présente un pouvoir hypertenseur double et la 3:5-diiodosynéphrine (DIS) quadruple de celui de (S); chez le chien (MIS) n'est guère plus actif que (S) tandis que (DIS) montre un effet tensionnel double. A flèche hypertensive égale, la durée de la réponse donnée par (DIS) est quatre fois plus allongée que celle de l'adrénaline. Des injections répétées n'entraînent pas de diminution de la réponse pressive et (MIS), mais surtout (DIS) en perfusion continue, produisent une élévation tensionnelle importante, soutenue, sans compensation ni hypotension secondaire; mêmes effets accompagnés d'hypoventilation pulmonaire et vasoconstriction rénale chez le chien amylé à surrénales exclues. On observe une inhibition augmentée en intensité et en durée de la motricité de l'intestin isolé de lapin proportionnellement à l'effet tenseur. Les qualités sympathomimétiques des iodosynéphrines sont donc caractérisées par une activité périphérique. Le rythme cardiaque, peu influencé par (MIS), est nettement ralenti par (DIS); atropine et section des vagues atténuaent ce ralentissement sans le supprimer. La consommation d'oxygène augmentée par (S) n'est pas modifiée par (MIS) et de faibles doses de (DIS); de fortes doses de (DIS) la diminuent chez le rat normal ou thyréoprive.

483b Pharmacology of Iodosynephrines. R. MICHEL, J. KLEPPING, R. TRUCHOT and H. TRON-LOISEL (France).

Iodination on 3 and 3-5 positions reinforces and prolongs the main sympathomimetic properties of Synephrine (S). In the rabbit, 3-iodosynephrine (MIS) shows twice the hypertensive potency and 3:5-diiodosynephrine (DIS) four times the potency of (S). At equal hypertensive levels, the duration of the response due to (DIS) is four times greater than that due to adrenalin. Repeated injections do not involve in a decrease the pressor response, and (MIS) and particularly (DIS) continues to produce a marked rise in blood pressure during continuous perfusion; this effect is constant without compensa-

tion or secondary hypotension. The same effect has been observed in the spinalized dog in which the adrenals had been excluded, in this case they were accompanied by pulmonary hypoventilation and renal vasoconstriction. The intensity and duration of inhibition of intestinal motility of the isolated rabbit intestine was found to be in proportion to the pressor effect. The sympathomimetic properties of iodosynephrine are thus characterized by peripheral effects. The cardiac rhythm, which was slightly affected by (MIS) was definitely slowed down by (DIS); atropine and vagus resection attenuate this effect without suppressing it. The oxygen consumption which is increased by (S) is not modified by (MIS) or by weak doses of (DIS); large doses of (DIS) decrease it in normal rats and thyroidectomized.

484 The Relation between Structure and Activity of Compounds with Vitamin K-like Activity. J. Lowenthal and J. A. MacFarlane (Canada).

All vitamin K-active compounds so far described are derivatives of 2-methyl-1:4-naphthoquinone and can be divided into 2 main groups: (1) Compounds like vitamin K_3 (2-methyl-1:4-naphthoquinone) which carry no substituent in the 3-position of the naphthalene nucleus; and (2) compounds like vitamin K_1 (2-methyl-3-phytyl-1:4-naphthoquinone) and vitamin K_2 (2-methyl-3-difarnesyl-1:4-naphthoquinone) which carry a substituent in the 3-position. Only compounds belonging to group 2 show significant antidotal activity against coumarin anticoagulant drugs. The present study of a series of vitamin K analogues shows that antidotal activity requires: (1) an intact or potential 1:4-naphthoquinone structure; (2) a relatively small substituent in the 2-position with inductive and polarizability effects which alter electron mobility in the naphthalene ring; and (3) a substituent in the 3-position which increases lipoid solubility.

Greatest specificity is shown by the substituent in the 2-position. By varying its size or electro negativity, analogues can be obtained which either show vitamin K-like activity, are inactive, or act as competitive antagonists. A similar relation is found for a series of substituted 1:4-benzoquinones. The data indicate that the size determines the interaction with receptors (affinity), while the electro negativity determines the biological response (intrinsic activity). The relative contribution of the two factors determines the overall effect.

485 Structure–Activity Relationships in a Series of Colchicine Analogues. R. Schindler (Switzerland).

The synthesis of colchicine by Eschenmoser *et al.*[1] has made available intermediates of this synthesis with chemical structures similar to that of colchicine. In order to study the biological activity of these compounds, their effects on *in vitro* cultures of murine mastocytoma cells, strain P-815-X_2, were analyzed. The cells were incubated in a medium containing various concentrations of the analogue to be tested, and after 24 hr, cell reproduction was measured. Colchicine inhibited cell reproduction at a concentration of $2 \cdot 10^{-5}$ mM. The specific metaphase-blocking action was followed by measurements of the proportion of metaphase cells and of anaphase-telophase cells 12 and 24 hr after addition of the analogue.

Removal of the acetyl group of colchicine resulted in a slight decrease of activity by a factor of approximately 2. Replacement of $-OCH_3$ by $-NH_2$ on the tropolone ring did not alter the activity appreciably. On the other hand, exchange of the positions of $-OCH_3$ and $=O$ on the tropolone ring caused a decrease of activity by a factor of nearly 100. All these compounds at inhibitory concentrations exhibited a metaphase-blocking action similar to colchicine. With a compound lacking the keto oxygen, no complete suppression of cell reproduction could be obtained at concentrations as high as 0·03 mM. The properties of a number of further colchicine analogues will be described.

(I am very grateful for the co-operation of Prof. A. Eschenmoser who generously supplied me with the colchicine analogues.)

1. Eschenmoser, A. *et al.* (1959). *Angew Chemie.* **71,** 637; 1961 *Helv. Chem. Acta*, **44**, 540.

486 The Metabolic Effects of Iodinated Salicylates in Normal, Hypo- and Hypermetabolic Rats. C. L. Gemmill and K. M. Browning (U.S.A.).

Various amounts of salicylate, 5-iodo-salicylate and 3:5-diiodo-salicylate were injected into normal and thyroidectomized rats and into rats made hypermetabolic by placing 3:3′:5-triiodothyronine in drinking water. The oxygen consumptions, rectal temperatures and pulse rates of these animals were determined. It was found that salicylate increased the metabolism of the normal and hypermetabolic rats but not the thyroidectomized animals. 5-iodo-salicylate increased the metabolism in the three groups. 3:5-diiodo-salicylate decreased the metabolism in the normal and hypo animals. The dosage of salicylate varied from 12·5–30·3 mg per 100 g body wt., 5-iodo-salicylate from 8·4–20·6 mg per 100 g of body wt. and 3:5-diiodo-salicylate from 1·0–20·6 mg per 100 g body wt. Results with 5-bromo-salicylate were comparable to those obtained with 5-iodo-salicylates. The effects of these compounds on rectal temperatures and pulse rates will be reported later.

487a Relations entre la Structure et l'Activité de Composés bis-Quaternaires Dérivés des Diythylaminoéthyl-théophylline et -théobromine. H. Morin (France).

A la suite d'expériences initiales faisant apparaitre les propriétés curarisantes de sels d'ammonium

quaternaires dérivés des diéthylaminoéthyl-théophylline et -théobromine, une étude plus vaste de composés bis-quaternaires obtenus par "doublement" des précédent a permis de vérifier que cette propriété croit avec l'allongement de la chaine polyméthylénique reliant les deux N: les dérivés décaméthyléniques présentent une activité maximum.

La curarisation exercée par ces composés est de type compétitif. L'étude plus particulière des dérivés décaméthyléniques isomères a montré qu'ils se différencient de la d-tubocurarine par un certain nombre de caractères intéressants: moins toxiques, d'action moins prolongée et plus rapidement reversible, pratiquement dépourvus d'actions secondaires (cardio-vasculaires, ganglioplégique) aux doses paralysantes. Cependant, ils ne semblent pas retarder l'atteinte respiratoire au cours de la curarisation, comme on pouvait l'espérer d'après les bases de depart.

Pour l'ensemble des composés étudiés, la structure des bases puriques intervient (volume des têtes cationiques) très problablement dans la *qualité* de l'action curarisante. *L'intensité* de cette dernière est réglée, en partie, par les radicaux fixes sur l'N, et surtout, par la longueur de la chaine carbonée intermédiaire. Celle-ci intervient pour mofigier les propriétés associées à la fonction ammonium quaternaire: son allongement a pour effet de diminuer fortement l'activité ganglioplégique relative et d'augmenter l'action inhibitrice à l'égard de la cholinestérase globulaire et, à un moindre degré, à l'égard de la cholinestérase plasmatique.

487b Relations between Structure and Activity of bis-Quaternary Derivatives of Diethylaminoethyl-theophylline and -theobromine. H. Morin (France).

Following preliminary experiments demonstrating curarizing properties of quaternary ammonium salts derived from diethylaminoethyl-theophylline and theobromine, a wider study of the bis-quaternary compounds obtained by "doubling" the preceding ones, showed that this property increases as the polymethylene chain linking the two N atoms is lengthened: decamethylene derivatives display maximum activity.

The curarization exerted by these compounds is of the competitive type. A more specialized study of the decamethylene isomer derivatives showed that they differ from D-tubocurarine in a number of interesting attributes: low toxicity, shorter and more easily reversible action, virtual absence of side-effects (cardiovascular, ganglion blocking) at doses producing paralysis. However, they do not appear to retard respiratory involvement during curarization as might have been hoped from the initial bases.

For all the compounds studied, the purine base structure affects (volume of cationic heads very probably the *quality* of the curarizing action. The *intensity* of the latter is regulated in part by the radicals joined to the N atom and above all by the length of the intermediate carbon chain. This factor helps to modify the properties associated with quaternary ammonium function: lengthening of the chain causes sharp reduction in the relative ganglion-blocking activity and increase in the inhibitory effect in relation to corpuscular cholinesterase and to a lesser degree plasma cholinesterase

488 Relation Between Chemical Constitution and Bronchodilator Activity in the Quinazolone Series. A. H. Amin, D. R. Mehta and S. S. Samarth (India).

A bronchodilator alkaloid vasicinone has been isolated in this laboratory from the leaves of *Adhatoda vasica* Nees[1] (N.O. Acanthaceae) and shown to be identical with 2:3(α-hydroxytrimethylene)-4-quinazolone obtained by the oxidation of vasicine with hydrogen peroxide.[2,3] Vasicinone has a bronchodilator action on normal guinea-pig lungs and a powerful action against histamine-induced bronchoconstriction. Vasicine has been shown in this laboratory to be a bronchoconstrictor. A CO-group in the 4-position of quinazolone therefore is indispensable for activity.

2:3-Trimethylene-4-quinazolone (I), 4-quinazolone (II), 4-pyrimidone (III) and α-naphthol (IV) have now been tested for bronchodilator activity. It is found that compounds I and II retain the activity, compound III is inactive and compound IV is a bronchoconstrictor. This shows that the 4-quinazolone molecule is characteristic of bronchodilator activity and that the α-hydroxytrimethylene ring in the 2:3 position is unnecessary for activity.

Alkyl, isoalkyl and allyl—groups in position 3 of 4-quinazolone showed higher activity than the parent 4-quinazolone. The addition of a benzene ring at the position 6:7 of 4-quinazolone led to a surprisingly high activity in 6:7-benzo-4-quinazolone, which was about 40 times stronger than 4-quinazolone. Substitution of alkyl- and isoalkyl-groups in the position 3 of this compound led to reduced activity.

The most important bronchodilators known are derivatives of phenylethylamines represented by adrenaline and those of xanthines represented by theophylline. The 4-quinazolone molecule therefore represents a new structure which has a powerful bronchodilator activity. Further work is in progress.

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2. Ghose, Krishna, Narang and Ray (1932), *J. Chem. Soc.*, **2,** 2740.
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489 Pharmacological Activity of 3-(10-11-dihydro-5H-dibenzo(a:Δ)cyclo-heptenyloxy)-tropane citrate (BS 6987). A. B. H. Funcke, D. Mulder, M. C. de Jonge, C. van der Stelt, A. F. Harms and W. T. Nauta (Holland).

The relationship observed between structure and pharmacological activity in a series of substituted benzhydryl aminoalkyl ethers led to the synthesis of a series of dibenzocycloheptene derivatives, the pharmacological properties of which were investigated. The compound BS 6987 proved to be a very potent antagonist of histamine and acetylcholine-induced spasms on the isolated guinea-pig gut.

Further investigations proved extremely good antagonism against the guinea-pig bronchospasm induced by inhalation of an acetylcholine, histamine, egg-albumen or serotonin aerosol.

On the strength of these results the drug was administered to patients suffering from bronchial asthma and chronic bronchitis. The first clinical results are favourable.

490 Structure–Action Studies on Spasmogenic Hydroxy-acids and Comparison with a Brain Acid. N. Ambache and M. Reynolds (United Kingdom).

Aqueous extracts of rabbit brain contain an active substance which contracts the atropinized hamster colon. It appears to be an irin-like unsaturated hydroxy-acid and has been purified by chromatography on SiO_2 columns, eluting with 0–30 per cent methanol in ether. Further properties will be described.

Structure–action studies on a series of natural and synthetic fatty acids have shown that the spasmogenic action on the hamster preparation depends on the presence of free carboxyl and hydroxyl groups and is nehanced by unsaturation, as shown in the Table, which also illustrates differences in fatty acid receptors between the guinea-pig ileum and the hamster (and rat) colon; the latter appear to be more sensitive detectors of unsaturated hydroxy-acids.

The following acids were inactive, illustrating further the dependence of activity upon structure: *Hamster* (1) 2-OH-stearic; (2) 10-OH-*trans*-2 decenoic; (3) (±)12-OH-dodec-10-ynoic; (4) (±) 6-OH-dodec-7-ynoic; (5) α- and β-kamlolenic; (6) 2-OH-nervonic; (7) Alternaric, 12-(5:6-dihydro-4-OH-6Me-2 oxypyran-3yl)-4:5-dihydroxy-3Me-9-methylene-12-oxododec-6-ene-5-carboxylic; (8) 7:8-dihydroxy-9-Me-3-methyl-eneundec-5-ene-1:7-dicarboxylic; (9) Saponified and unsaponified (a) δ-dodecalatone, (b) Δαβ- and Δγδ-δ-hexenolactones, and (c) β me-Δβγ-δ- hexenolactone; (10) *p*-hydroxy- and 3-4-dihydroxy-benzoic; (11) *trans* -ximenyic (the *cis* was active). *Guinea-pig* (12) $\Delta^{5,\ 8,\ 11,\ 14,\ 17}$ eicosapentaenoic (virtually inactive); (13) Saponified lactones as in (9); (14) Alternaric; No. 8 was weakly active.

Table 1

Fatty acids	Hamster colon	Rat colon	Guinea-pig ileum
9-octadecenoic	—	—	—
3-octadecenoic (Gabr, 1956)		—	+
(+) 12-OH-*cis*-9-octadecenoic	$+(10^{-6})$	+	—
Methyl -12-OH-*cis*-9-octadecenoate	—		
(+) 12-OH-*trans*-9-octadecenoic	$+(10^{-6})$		
(±) 12-OH-9-octadecynoic	$+(10^{-6})$		
12-OH-stearic	— (5×10^{-6})		
(+) 9-OH-*cis*-12-octadecenoic	+ (5×10^{-6})		

Gabr, Y. (1956), *Brit. J. Pharmacol.*, **11**, 93.

491 Antagonism of Adrenaline and Histamine by Some Closely Related Halogenoalkylamines. J. D. P. Graham and M. A. Karrar (United Kingdom).

The hydrolysis products of dibenamine and phenoxybenzamine have no antagonism to the motor actions of adrenaline, noradrenaline, histamine or 5-hydroxytryptamine. The active chemical species is held to be the cyclized form or imine ion. When the β carbon of the ethylamine chain is substitute with a phenolic group the structure resembles that of adrenaline. Specific antagonism is much increased, but its duration is short. The block is non-competitive. The alcohol of such a compound is a potent local anaesthetic but not an antagonist of the amines. If the phenyl is substituted by *p* or *o*-Br or CH_3 or both, the intensity of anatagonism is again increased but duration is very brief and the nature of the block is non-competitive. The alcohols, which closely resemble dichloro-isoprenaline or its analogues are competitive antagonists of histamine. They are weak agonists of motor activity and cause an atropine-sensitive spasm of gut.

492 An Analysis of the Effect of Pharmacological Agents on Conduction of Stimulation in Different Sections of the Neuro-muscular Junction with the aid of Multi-channel

Intra-cellular Micro-electrodes. A. I. Shapovalov (U.S.S.R.).

The resting potential, the local and spreading potentials of the muscle fibre, the potentials of the en plate and the spontaneous miniature potentials were all recorded in experiments with a neuro-muscular preparation of the frog. The agents tested were added to the solution bathing the muscle, or were introduced ionotophoretically either from without or else from inside the cell through multi-channel (2–6 channels) micro-electrodes. In the last case one of the channels of the microelectrode served to neutralize the intra-cellular difference of potentials, another one for polarization or direct stimulation, and 2–4 others served to administer various pharmacological agents.

As a result of the above experiments it became clear that the different pharmacological agents block the neuro-muscular transmission by acting on various segments of the synapse: the presynaptic nerve endings (CoC12, CdC12), the subsynaptic receptor membrane (muscle relaxants—piplacin, succinylcholine, decamethonium), the electro-excitable membrane of the muscle fibre (barbiturates, Ca ions). Some substances (Novocaine) will depress transmission in all the links of a synaptic chain.

The paper describes multiple responses appearing as a result of action by pharmacological agents on the presynaptic nerve endings by the cholino-receptive and the electro-excitable membrane.

The paper discusses the question of pharmacological and physiological peculiarities of the membrane of the muscle fibre. A definite resemblance was found to exist between certain reactions (alleviation, desensitization) of the subsynaptic cholinoreceptive membrane of the end plate and the electro-excitable muscle fibre membrane that surrounds it.

493 Effect of Depolarizing Drugs on the Neuro-muscular Transmission in Submaximal and Supramaximal Stimulation. G. Schmidt, W. D. Erdmann and H. Dal Ri (Germany).

Each single synaptic unit gives an "all or none" reaction. The graduated response of the target-organ to presynaptic stimuli is therefore mainly achieved by variation of the number of synaptic units, taking part in the transmission. There is no linear relationship between presynaptic stimulation and the active performance of the target-organ. Only if at least 25 per cent of the synaptic units take part in the transmission is a stimulus followed by a response. Maximal reaction is already achieved when 75 per cent of the synaptic units participate in the transmission. This explains the phenomenon described by Vulpian. A partial blocking of neuro-muscular transmission by curarine abolishes spontaneous motility, while supramaximal stimulation of the nerve still results in a full response of the muscle.

Apparently, these conditions apply not only for membrane stabilizing muscular relaxants but also for depolarizing agents, including anticholinesterases. One might expect that an increased accumulation of acetylcholine results in a neuro-muscular paralysis of the type of the Wedensky-inhibition. This inhibition should be more pronounced if the number of stimulated synapses is increased. Experiments with depolarizing agents, such as neostigmine, on the rat diaphragm *in situ* demonstrate, however, that although the neuro-muscular block for physiological impulses of the phrenic nerve is complete, supramaximal stimulation of the nerve results in a full muscular reaction. The assumption that there is a variable sensitivity of different neuro-muscular synapses for depolarizing agents would offer an explanation for these results. If part of the endplates does not succumb to the Wedensky-inhibition, a supramaximal stimulation may pretend that the neuro-muscular transmission is not altered.

494 The Pharmacology of Substances Related to Choline. M. J. Rand and W. C. Bowman (United Kingdom).

Recently, Bowman *et al.*[1] showed that the triethyl analogue of choline (*N*-triethyl ethanolamine, TEC) produced a neuromuscular blockade resembling that produced by hemicholinium. The neuromuscular blockade is probably produced by inhibition of the synthesis of acetylcholine at motor nerve endings. A series of related compounds have now been tested for this action, as well as for a variety of other pharmacological actions.

1. Bowman and Rand (1961), *Lancet*, **1**.

495 The Neuromuscular Blocking Action of the Triethyl Analogue of Choline. W. C. Bowman and M. J. Rand (United Kingdom).

The triethyl analogue of choline (*N*-triethyl ethanolamine, TEC) produces a partial neuro-muscular block in muscles which are stimulated rapidly, but not in slowly-stimulated muscles. When injected into conscious animals, TEC produces muscular weakness after exercise. The actions of TEC are probably produced by reducing the rate of synthesis of acetylcholine, so that the neuromuscular block and muscle weakness are results of depletion of acetylcholine from motor nerve endings. The effects of TEC can be reversed by choline, and the structure of TEC is such as to suggest that it is an antimetabolite of choline which specifically interferes with its acetylation.

496 Certain Aspects of Pharmacology of Neuromyal Cholinergic Receptor. A. G. KARCZMAR, K. C. KIM and K. KOKETSU (U.S.A.).

This summarizes several lines of research on cholinergic receptor, carried out during recent years as well as presents newer data throwing additional light on the pharmacology of the receptor and on its flexibility. Firstly, many data from these and other laboratories suggest that cholinergic and cholinesterase receptors are not identical. Following administration of anticholinesterases in doses many times those sufficient to inhibit all cholinesterases, responsiveness to acetylcholine remains unimpaired and even function may return to normal. It was early suggested that quaternaries may exhibit both receptor and anticholinesterase action; it was demonstrated indirectly in these laboratories that one effect may preclude the other. This suggests competition for the agent between the two receptors. Secondly, quaternaries may exert facilitatory effects at the receptor by a mechanism different from that of anticholinesterase action. In contradistinction to the latter, facilitation produces augmentation without prolongation of acetylcholine depolarization and of endplate potential. Finally, even prolonged depolarization appears, in certain conditions, as a pure excitatory process not concomitant with blockade.[1, 2] The latter may appear upon termination of depolarization and depends on receptor desensitization; this desensitization differs in certain respects from competitive blockade, and it may terminate spontaneously even in the presence of acetylcholine. It is speculated that the interplay between desensitization and restoration of sensitivity is the property of cholinergic receptor which underlies recovery of function after anticholinesterase treatment, and may contribute to the physiological functioning of the receptor.

1. PATON and ZAIMIS (1957), *J. Physiol*, **112,** 311.
2. KARCZMAR (1961), *Symp. on Bioelectrogenesis*.

497 Action of Pharmacological Drugs on the Miniature End-plate Potentials in Neuromuscular Junctions. E. A. LIBERMAN and I. M. GLAGOLEVA (U.S.S.R.).

Past experiments have not given an answer with any certainty to the question: is a quantum of acetylcholine released spontaneously from the end plate and is a single miniature potential produced by one or many molecules.

To solve this question, the action of cholinesterase on miniature end plate potentials has been studied. If the quantity of acetylcholine had consisted only of one molecule, the cholinesterase might have destroyed it completely and this would lead to a decreased frequency of miniature potentials. There were no changes in their amplitude.

The changes in amplitude of miniature potentials induced by cholinesterase indicate a multi-molecular composition of acetylcholine and allow an approximate calculation of its amount (10^3 molecules for the neuromuscular junction in m. sartorius of frogs).

Data concerning the action of atropine, adrenaline, barbamyl, dyplacin, novocaine, nicotinic acid, strychnine, urethane and phosphacol on the parameters of miniature potentials will be presented.

498 Contracture of the Isolated Denervated Rat Diaphragm by Adrenaline. K. D. BHOOLA and M. SCHACHTER (United Kingdom).

Denervated skeletal muscle has been known for a long time to become hypersensitive to ACh.[1] It is now known that this is associated with the fact that whereas in the normal muscle fibre ACh produces membrane depolarization only when applied to the end-plate region, in a denervated muscle the entire muscle membrane becomes sensitive to ACh.[2, 3]

Orbeli (1923) demonstrated that the application of adrenaline or sympathetic nerve stimulation to skeletal muscle increased the twitch in response to motor nerve stimulation. Despite much analysis since then, no evidence has been presented that adrenaline itself will contract skeletal muscle.

The present experiments demonstrate that adrenaline (10^{-6}–10^{-7} g/ml) regularly produces a contracture of the denervated (10–25 days), isolated rat diaphragm when tested in Tyrode solution at 34°–37°C. The contracture produced by adrenaline was slower and more protracted than that produced by ACh and unlike that of ACh was unaffected by eserine (5×10^{-6} g/ml) or by curare (10^{-6} g/ml). The contractures produced by ACh and by adrenaline were greatly reduced or abolished in the absence of calcium.

The denervated rat diaphragm is rather specific, responding only to cholinesters, quaternary ammonium compounds, and to adrenaline. It fails to respond to 5-HT, histamine, bradykinin, angiotensin and pilocarpine.

These observations raise the possibility of a physiological role of adrenaline in skeletal muscle.

1. CANNON, W. B. and ROSENBLUETH, A. (1949), *The Supersensitivity of Denervated Structures*. Macmillan, New York.
2. AXELSSON, J. and THESLEFF, S. (1957), *J. Physiol.*, **149,** 178.
3. MILEDI, R. (1960), *J. Physiol.*, **151,** 1.

499 The Action of Cholinolytics and Adrenomimetics on Tonic Muscles. L. G. MAGAZANIK, M. J. MICHELSON, E. K. ROSHKOVA and I. A. SHUMOVA (U.S.S.R.).

According to the data obtained by Ginetsinsky *et al.*(1937–1949) it was shown that the contractor of

tonic muscle fibres of Cyclostomata, amphibia and birds, induced by acetylcholine and cholinomimetics or by indirect excitation, is abolished by cholinolytics much more readily, than the reaction of tetanic fibres.

The contracture of tonic fibres is abolished not only by curare-like drugs but also by other cholinolytics which lack curare-like activity (atropine, benactyzine, parpanit, hexamethonium and others).

The contracture of cock's and pigeon's tonic muscle induced by acetylcholine and some leptocurare is abolished by epinephrine. With i.a. injection epinephrine was effective in the dose 0·005 μg. Under some experimental conditions a biphasic effect of epinephrine can be observed: after initial inhibition the contraction can be enhanced. When stimulated indirectly for 5–20 min (rate 50–100 per sec) the cock's gastocnemius develops a contracture which is maintained only by tonic fibres. This contraction is also abolished by epinephrine.

In tonic fibres of frog muscles true and pseudocholinesterase was histochemically revealed only in the multiple end plates. In rabbit's gastrocnemius 26 days after denervation the cholinesterase is revealed not only in the end plates, but throughout the length of the fibres.

In early chicken embryogenesis (before the thirteenth day of incubation) the cholinesterase is distributed diffusely. Until the sixteenth to nineteenth days of incubation, the cholinesterase accumulates in the end plate regions.

500a Transmission neuromusculaire et Tetrodotoxine. J. Cheymol et F. Bourillet (France).

Dans la transmission neuromusculaire plusieurs chainons interviennent: fibre nerveuse; acétylcholine; plaque motrice-fibre musculaire. Chacundes ces chainons peur-être bloqué, entrainant ainsi une paralysie motrice.

La tétrodotoxine, extrait des viscères du poisson Fugu (Tétraodontinés) très recherché par les gourmets, au Japon surtout, est responsable de nombreux empoisonnements. Un des symptômes principaux est une paralysie motrice, entrainant un arrêt respiratoire.

L'analyse du mécanisme de cette action paralysante conduit aux conclusions suivants:

(1) l'action périphérique dela tétrodotoxine est certaine puisque celle-ci inhibe les contractions de préparations neuromusculaires isolées ou séparées des centres nerveux. Toutefois, nos résultats ne nous permettent pas de rejeter une action supplémentaire d'oridinecentrale;

(2) ce n'est pas une curarisation. Comparée sur plusieurs tests pharmacologiques aux substances curarisantes classiques (curarimimétiques et acétylcholinomimétiques), l'action de la toxine se montre complexe et atypique: augmentation de l'effet paralysant de la d-tubocurarine comme par la succinyldicholine: absence d'antagonisme par les anticholinestérasiques, quelle que soit l'espéce animale; pas de paralysie flasque ni de contracture sur le poussin; pas d'action contracturance ou inhibitricesur le rectus de grenouille.

La diminution progressive du potentiel de plaque motrice est toutefois constatée montrant que l'action que l'action s'effectue soit au niveau de la plaque soit sur la fibre nerveuse ou ses terminaisons.

(3) la toxine exerce une action inhibitrice directe de la fibre musculaire. La stimulation électrique directe d'une fibre musculaire innervée ou dénervée n'entraine plus de contraction après action de la toxine.

(4) la conduction de l'influx nerveux est bloquée sur la préparation sciatique-gastrocnémien de grenouille, de faibles concentrations de toxine bloquent la conductione de l'influx nerveux moteur. Cet effet pourrait expliquer la baisse du potentiel de plaque motrice.

(5) l'acétylcholine ne forme pas *in vitro* de combinaison inactive avec la toxine.

(6) empêche-t-elle la biosynthèse ou la libération du transmetteur physiologique de l'influx moteur? Des résultats expérimentaux (augmentation d'amplitude des contractions par augmentation de fréquence, sensibilité diminuée d'une préparation neuromusculaire *in situ* aux injections proches d'acétylcholine) fournissent des preuves indirectes peu favorables à un tel mécanisme.

En résumé: Action paralysante complexe atypique mettant en jeu au moins deux phenomènes: inhibition directe de la fibre musculaire, blocage de la conduction de l'influx nerveux moteur.

500b Neuromuscular Transmission and Tetrodotoxin. J. Cheymol and F. Bourillet (France).

In neuromuscular transmission several links come into play: nerve fibre; acetylcholine; motor plate; muscle fibre. Each of these links may be blocked with ensuing motor paralysis.

Tetrodotoxin, extract of the viscera of the fish Fugu (Tetraodontines) greatly sought after by gourmets, especially in Japan, is responsible for many cases of poisoning. One of the chief symptoms is motor paralysis involving respiratory arrest.

Analysis of the mechanism of this paralysing action leads to the following conclusions:

(1) the peripheral action of tetrodotoxin is beyond doubt since it inhibits contractions of isolated nerve-muscle preparations of those separated from the nerve centres, but our results do not allow dismissal of a supplementary central effect.

(2) this action is not curarization as is shown when it is compared in several pharmacological tests with classical curare-like substances (curarimimetics and acetylcholine-like substances), when the action of the toxin is complex and atypical: increasing the paralysing effect of D-tubocurarine as for succinyldicholine; absence of antagonism with anticholinesterases, whatever the animal species; no flaccid paralysis or contractures in the chick; no contractile or inhibitory effect on the frog rectum.

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The progressive fall in the potential of the motor plate was, however, noted, showing that the action is either at the level of the plate or on the nerve fibre or its endings.

(3) the toxin exerts a direct inhibitory effect on muscle fibre, as direct electrical stimulation of an innervated or denervated muscle fibre no longer produces contraction after the effect of the toxin.

(4) the conduction of the nerve inflow is blocked in the frog sciatic-gastrocnemius preparation; weak concentrations of the toxin block the conduction of motor nerve inflow and this effect may explain the drop in potential at the motor plate.

(5) *in vitro*, acetylcholine does not form an inactive complex with the toxin.

(6) does it prevent biosynthesis or liberation of the physiological transmitter of motor inflow? Experimental results (increased amplitude of contractions with rise in frequency, reduced sensitivity of a nerve muscle preparation *in situ* near to the injection of acetylcholine) furnish indirect proof providing little support for such a mechanism.

Summary: Tetrodotoxin has a complex and atypical action bringing into play at least two phenomena: direct inhibition of the muscle fibre and blocking of the conduction of motor nerve inflow.

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501 Effect of Adrenaline and Noradrenaline on Tension Developed in Cat's Soleus and Tibialis Anterior Muscles Stimulated at Various Frequencies. I. Jurna and W. Rummel (Germany).

In cats anaesthetized with nembutal, soleus and tibialis anterior muscles were isolated. The peripheral ends of the dissected motor nerves were stimulated at 10, 15, 20, 30 and 40 (50) stimuli per sec (sub- and supramaximal shocks). Various amounts of extension were applied to the muscles previous to stimulation. Tension was recorded isometrically. In tension-frequency diagrams the tension of the soleus muscle followed an exponential curve, which was steepest and nearly linear from 10 to 20 per sec. A plateau was reached at 40 or 50 per sec. With tibialis anterior no plateau was reached up to the maximal frequencies tested; tension increase with stimulation frequency was largest within the range from 20 to 30 or 40 per sec. Adrenaline and noradrenaline caused a reduction of muscle tension in soleus and an increase in tibialis anterior, as has been previously reported by Bowman *et al.*[1] With supramaximal stimulation, the decrease of tension in the soleus muscle following intravenous injection of adrenaline (0·5–1·0 μg/kg) or noradrenaline (1·0 μg/kg) was most pronounced at lower frequencies (10–20 per sec); at higher frequencies both amines had little or no effect. With submaximal strength the decrease in tension was seen at all frequencies. In tibialis anterior adrenaline and noradrenaline (3·0–5·0 μg/kg) caused the largest increase in tension at 20 and/or 30 per sec.

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1. Bowman and Zaimis (1958), *J. Physiol.*, **144**, 92.

502 The Influence of Various Pharmacological Agents on the Rate of Rigor Mortis in Skeletal Muscles. V. M. Karasik (U.S.S.R.).

The report is a review of works, performed by the author and his collaborators (I. V. Markova, E. V. Moreva, A. I. Podesnaya, L. I. Tank).

A method to register the rate of development of rigor of the tail in mice and rats, was elaborated. After administration of the drug decapitation (stopping the oxidative phosphorylation whereas the glycolytic phosphorylation lasts for a some time) was carried out and time of rigor was determined.

The rigor is distinctly accelerated after 2:4-dinitrophenol (DNP), less distinctly after sodium azide and least of all after fluoride, monoiodacetate and iodacetamide (all substances in sublethal doses).

After sublethal doses of DNP, the rigor appeared when the ATP content in muscles was relatively high. It is possible that DNP disturbs such transphosphorylation process, which maintains the native state of contractile protein.

Injection of epinephrine, which promotes the phosphorylation, restores the time of rigor, accelerated by DNP, but it does not influence the time of rigor in normal animals.

The rigor is retarded after injection of insulin, promoting the glucokinase reaction, and after adrenalectomy, when the inhibitory influence of glucocorticoids on the glucokinase reaction was abolished. The injection of the adrenal cortex preparation "Cortin" (but not cortisone or DOCA) in adrenalectomized animals restores the time of rigor.

The rigor in newborn mice, rats, rabbits and cats) (but not in guinea pigs) appeared later than in adult animals. The latter must be connected with failure of glucocorticoids secretion in the newborn and probably with a longer native state of contractile protein.

503 Influence of Narcotic Drugs upon the Reactiveness of Skeletal Muscles to Acetylcholine and Potassium Ions. E. V. Moreva (U.S.S.R.).

Narcotic drugs reduce or even abolish contracture of the m. rectus abdominis of the frog brought about by potassium. At the same time they not only do not reduce acetylcholine contractures, but, in many cases, even intensify them. Analogous results are obtained in experiments on the isolated diaphragm of the rat.

The author assumes the presence of some structural component in the skeletal muscle which is depressed by narcotic drugs and possesses a more

pronounced selective reaction to potassium than to acetylcholine.

Intensification of acetylcholine induced contracture under the action of narcotic drugs cannot be ascribed to cholinesterase depression, as has been shown in a former investigation.

504 Effect of Enzymes on Isolated Rat Mast Cells. R. KELLER (Switzerland).

The effect of a number of enzymes on mast cells isolated from the peritoneal cavity of the rat was investigated by phase contrast microscopy at 37°C as well as by quantitative determination of the percentage of damaged cells after incubation of normal cells for 4 min at 37°C. The results of these investigations are in good agreement with those obtained on isolated rat mesenteries. Thus they confirm the observations of Högberg.[1] The significance of these findings is briefly discussed.

1. HÖGBERG and UVNÄS (1957), *Acta Physiol. Scand*, **41**, 345.

505 Proteolytic Enzyme and Tissue Activator Associated with Canine Mast Cell Tumour. N. ENDE and J. V. AUDITORE (U.S.A.).

In the past few years, the mast cell has been shown to be a veritable pharmacological storehouse. Recently the authors reported that the mast cell of the dog and human is associated with an unidentified proteolytic enzyme. Preliminary studies on the intracellular distribution of this proteolytic enzyme revealed its presence in all compartments of the cell with greatest quantities in the mitochondrial fraction. Recent studies[1] have shown that 64 per cent of the total heparin is also present in the mitochondrial fraction. Tissue activator of the fibrinolytic enzyme system was also found associated with the mast cell tumour. The microsomal fraction contained most of this activity.

Additional studies on the hydrolysis of amino acid esters employing the Warburg technique show that the dog mast cell tumour hydrolyzes *p*-toluenesulphonyl arginine methyl ester (TAME) at an appreciable rate (7·2 µl CO_2/10 min /0·3 ml homogenate) at pH 7·4. Five other different types of dog tumours failed to hydrolyze TAME tested in a similar system. Dog mast cell tumour homogenate was also found to hydrolyze acetyl-L-tryptophan ethyl ester. The hydrolysis of this ester, however, was not believed to be a specific action of the homogenate of mast cells as different types of dog tumours also hydrolyzed this same amino acid ester.

1. KORN (1959), *J. Biol. Chem.*

506 Uptake and Metabolism of Amines by Mast Cells in Culture. J. P. GREEN and S. M. DAY (U.S.A.).

Two strains of a murine mast cell tumour (P-815), maintained in culture for more than 2 years, retain the capacity to take up and to decarboxylate histidine and 5-hydroxytryptophan (5-HTP) to their respective amines. The cells are able also to concentrate pre-formed histamine and 5-hydroxytryptamine (5-HT). The rates of turnover of exogenous histamine differs from the turnover-rate of endogenously formed histamine. Both strains of neoplastic mast cells are able to take up 3-hydroxytyramine and tryptamine, although to a lesser extent than histamine or 5-HT, but no evidence could be obtained that either 3-hydroxytyramine or tryptamine are normally present in these cells. Incubation of these cells with tryptophan results in the formation of 5-HTP and 5-HT and only small amounts of 5-hydroxyindole acetic acid; no other metabolic products of tryptophan or of 5-HT could be detected. No evidence could be obtained that either endogenous or exogenous histamine is further metabolized.

507 Response to Histamine in Mental Patients before and after Tranquilizer Therapy. J. LEBLANC (Canada).

The skin of mental patients was shown to contain fewer mast cells than normally. However, after one month's tranquilizer therapy the number of these cells increased significantly. The cutaneous response to histamine and tubocurarine was much smaller in mental patients than in normal subjects. However, patients who were sufficiently improved to be released after one month's tranquilizer therapy, were more sensitive to histamine at the end of this period than they were originally. Unimproved patients showed no such increase in histamine response although after six month's therapy they were shown to be less tolerant to histamine. Schizophrenics as a group showed a better response to tranquilizers, as judged by histamine sensitivity, than any other group of mental patients studied.

508 The Action of Adrenal Cortical Hormones on Histamine Metabolism. J. M. TELFORD and G. B. WEST (United Kingdom).

Injections of glucocorticoids reduce the histamine and 5-hydroxytryptamine contents of rat skin and small intestine but increase the levels of these amines in the pyloric stomach.[1] Experiments have been carried out to determine if these changes are due to alterations in the rate of formation of the amines.

The histidine decarboxylase activity of rat tissues was estimated using a modification of Waton's method[2]; with this technique, the most potent sources of the enzyme are the liver and pyloric stomach.[3] Treatment with glucocorticoids in-

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hibited the liver enzyme but increased the activity in the pyloric stomach, and similar changes were found after exposure to cold and after large doses of potent histamine liberators. On the other hand, the activity of tissue 5-hydroxytryptophan decarboxylase was not significantly changed by any of these procedures.

The effectiveness of glucocorticoids in allergy and inflammation thus may be due to inhibition of the rate of histamine formation. The steroids most active in altering tissue histidine decarboxylase activity are those possessing greatest glucocorticoid activity. For example, triamcinolone, fludrocortisone and 2-methylfludrocortisone are more active than prednisolone and cortisone, and deoxycortone (a mineralocorticoid) is inactive. As the adrenal cortical secretion in the rat is predominantly glucocorticoid and as the effects of its release mimic those of exogenous glucocorticoids, the biosynthesis of histamine may be controlled by the adrenal cortex.

1. Telford, J. M. and West, G. B. (1960), *Brit. J. Pharmacol.*, **15,** 532.
2. Waton, N. G. (1956), *Brit. J. Pharmacol.*, **11,** 119.
3. Telford, J. M. and West, G. B. (1961), *J. Pharm., Lond.*, **13,** 75.

509 The Action of Dextran in Rats. G. B. West (United Kingdom).

In 1937, Selye[1] reported that the primary injection of egg white into rats produces oedema of the face, tongue and paws, hyperaemia and pruritus, but the symptoms are never so severe as to produce death. Dextran also produces the effect which is mainly due to the release of 5-hydroxytryptamine.[2] The mechanisms by which rats become supersensitive to dextran when treated with hormones have now been investigated.

Treatment with thyroxine, tri-iodothyronine, insulin, chlorpropamide and 5-hydroxytryptophan, or the operation of adrenalectomy, potentiate the dextran response so markedly that more than half of the rats die. The cause of death in all cases is internal haemorrhage resulting probably from accumulation of released 5-hydroxytryptamine and histamine, but the glucose transport system is also involved since the supersensitivity is greatly reduced by injections of glucose, alloxan, glucocorticoids, adrenaline and thiouracil and by thyroidectomy. As shown by Archer,[3] dextran combines with γ-globulin of the serum to give a potent releaser of both histamine and 5-hydroxytryptamine, whilst an elevated blood sugar level reduces this combination. It is possible, however, that other factors are involved since a proportion of control Wistar rats (about 19 per cent) fail to give this anaphylactoid reaction, even when subjected to treatments which usually produce supersensitivity.

1. Selye, H. (1937), **21,** 169.
2. Parratt, J. R. and West, G. B. (1957), *J. Physiol.*, **139,** 27.
3. Archer, G. T. (1959), *Nature (Lond.)*, **184,** 1151.

510 Concomitant Release of Histamine and 5-Hydroxytryptamine from Rat Mast Cells. N. C. Moran, B. Uvnäs and B. Westerholm (Sweden).

Rat mast cells have been shown to contain both histamine[1] and 5-hydroxytryptamine[2]. The following experiments were performed in order to compare the patterns of release of the two amines from peritoneal mixed cell suspensions and isolated peritoneal mast cells.

Time course of release for both amines was extremely rapid, the entire release occurring within 15 sec based upon experiments with perfused cells. Over a range of 0·1–2·5 μg/ml of 48/80, the dose response curves for release of histamine and 5-hydroxytryptamine were linear and parallel. Chemical inhibitors (SH inhibitor) and ninhydrin (NH_2 inhibitor) produced comparable inhibition of the 48/80-induced release of both amines. Dinitrophenol inhibited release only in a glucose-free medium. Cooling of the cells to 0·5°C caused reversible inhibition, and heating to 47·5°C irreversible inhibition of amine release. Release of both amines was produced by phospholipase A, n-decylamine and distilled water but not by phospholipase D and trypsin.

It is concluded that 5-hydroxytryptamine is released in the same fashion as histamine in response to certain histamine releasing agents.

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511a Influence de l'Ion Calcium sur les Effets Pharmacologiques de l'Histamine, de la 5-Hydroxytryptamine, et de la Reaction Anaphylactique, in vitro. H. V. Huidobro (France).

Sur l'iléon isolé de cobaye plongé dans du tyrode dépourvu de $CaCl_2$, l'histamine à la dose de 0·02 μg/ml, ne produit aucune contraction. L'addition ultérieur de 40 μg/ml de $CaCl_2$ provoque une résponse qui correspond à la dose d'histamine déjà présente dans le bain. Même si l'addition de calcium est rétardée jusqu'à 10 minutes, la contraction due à l'histamine reste inchangée.

L'addition de 5-hydroxytryptamine à la dose de

0·2 μg/ml au bain de tyrode sans calcium reste sans effet. Si on ajoute 40 μg/ml de $CaCl_2$ 70 sec aprés, on n'observe aucune action contractile. Toutefois, l'organe respond normalement à l'histamine.

L'inhibition de l'effet de la 5-hydroxytryptamine ne semble pas être due à une destruction de l'amine: si le liquide du bain renfermant l'iléon de cobaye desensibilisé à la 5-hydroxytryptamine est transvasé dans une cuve contenant un autre iléon de cobaye, ce dernier se contracte avec une amplitude correspondant à la dose de 5-hydroxytryptamine du bain.

La réaction anaphylactique sur l'iléon de cobaya sensibilisé ne se produit pas non plus en l'absence de calcium. L'addition de 40 μg/ml de $CaCl_2$ provoque, aprés un temps de latence d'environ 20 sec, une contraction maximale de l'intestin.

Cependent, l'absence de calcium n'entrave pas la combinaison antigène-anticorps: si aprés l'introduction de l'antigène on pratique plusieurs lavages, l'addition de $CaCl_2$ révèle encore la réaction anaphylactique de l'organe.

Etant donné le temps de latence observé entre la récalcification et la réponse de l'organe, le calcium interviendrait dans les premiers stades de la réaction anaphylactique.

511b The Influence of the Calcium Ion on the Pharmacological Effects of Histamine, 5-Hydroxytryptamine and on the Anaphylactic Reaction in vitro. H. V. Huidobro (France).

Histamine in a dose of 0·02 μg/ml does not cause any contraction of the isolated guinea-pig ileum, immersed in a tyrode bath from which $CaCl_2$ has been excluded. The subsequent addition of 40 μg/ml of $CaCl_2$ causes a response which corresponds to the dose of histamine already present in the bath. Even if the addition of calcium is delayed by up to 10 min, the contraction due to histamine remains unchanged.

The addition of 5-hydroxytryptamine in a dose of 0·2 μg/ml to the tyrode bath without calcium has no effect. If 40 μg/ml of $CaCl_2$ are added 70 sec later, no contractile effect is observed. However, the organ responds normally to histamine.

Inhibition of the effect of 5-hydroxytryptamine does not seem to be due to the destruction of the amine: if the liquid in the bath containing the guinea-pig ileum which has been desensitized to 5-hydroxytryptamine is transferred to another tank containing another guinea-pig ileum, the latter contracts with an amplitude which corresponds to the dose of 5-hydroxytryptamine in the bath.

The anaphylactic reaction of the sensitized guinea-pig ileum cannot be evoked either in the absence of calcium. The addition of 40 μg/ml of $CaCl_2$ provokes a maximal contraction of the intestine after a latent period of about 20 sec.

However, the absence of calcium does not impede the antigen-antibody combination: if the introduction of the antigen is followed by several washings, the addition of $CaCl_2$ results in an anaphylactic eraction by the organ.

Given the latent period which was observed between recalcification and the response by the organ, calcium must enter into the first stages of the anaphylactic reaction.

512 Atropine – Histamine Interaction. G. D. Maengwyn-Davies, S. Eldar, S. I. Oroszlan and M. Wurzel (U.S.A.).

We have found that atropine, upon ageing or ultra-violet irradiation, shows an increase in enol content, as determined qualitatively by ferric chloride. Such atropines give a complex with protein, amino-acids and simple amines, which can be shown spectrophotometrically by an absorption peak at or near 295 mμ, as do non-aged and non-irradiated atropines at much higher concentrations. Atropine can be determined quantitatively at one of its absorption peaks at 258 mμ. Enolized atropine shows an increase in absorption with increase in pH at this absorption peak, as well as a small new absorption peak at 264 mμ. Histidine was the most reactive amino acid studied; l-,N-methylhistidine still gives the reaction. Histamine also shows complex formation with a pH optimum at 6·5. This complex forms stoicheiometrically between two moles of atropine and one mole of histamine. Complex formation is competitively inhibited by cysteine or homocysteine. The inhibition is partially reversed by *p*-chloromercuribenzoate. *S*-methylcysteine shows complex formation. Thus, inhibition is due to the free SH- group, which, as a strong electron acceptor, might promote keto-tautomerism. The guinea-pig ileum was employed to demonstrate atropine–histamine complex formation. Atropine–histamine complex was far less active in contracting the organ than a freshly-made mixture of atropine and histamine. It is suggested that atropine–histamine antagonism may be due in part to complex formation.[1]

[1] (Supported by Grant B-1828 (C-2), National Institute for Neurological Diseases and Blindness, U.S.P.H.S.)

513 Spike Generation by Intestinal Smooth Muscle during Complete Absence of Sodium in the External Medium. E. Bülbring and H. Kuriyama (United Kingdom).

Action potentials were recorded with intracellular electrodes from spontaneously discharging cells of taenia coli. Experiments in completely Na-free solutions (Na-buffers replaced by K-buffers) confirmed and extended previous observations.[1, 2, 3, 4] Sucrose decreases membrane activity which ceases after 20–40 min. Choline depolarizes the membrane and rapidly abolishes spike generation but in the presence of atropine, choline increases membrane activity and spikes do not cease for 30 min or more. Lithium, after transient depression, increases discharge frequency. Membrane poten-

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tial and spike height are slightly decreased, but spike generation continues for 1 hr or more. This confirms results obtained with lithium and hydrazine using the sucrose gap method. Tetraethylammonium transiently increases spike height, then it depolarizes, reduces spike height and prolongs the spike duration 100 times. Within 5 min spike discharge ceases. Tris(hydroxymethyl)aminomethane increases spike frequency and sometimes spike height. Spike generation continues for 30–60 min.

Goodford and Hermansen[5] observed that more than 95 per cent of the muscle sodium is exchanged within 5 min in ^{24}Na solution. Decreased membrane activity in sodium-free solutions may not be primarily due to sodium lack but to a change of membrane permeability or of metabolic processes by the substances used as substitutes. Spike generation in taenia coli may be independent of sodium. On the other hand, the substitutes used may maintain activity for short periods by acting in place of sodium, or spike generation might be maintained by leakage of bound sodium from an extracellular or intracellular element.

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514 Effects of Nitrate on the Electrical and Mechanical Activity of Intestinal Smooth Muscle. J. Axelsson (United Kingdom).

Replacement of chloride with nitrate increases the frequency of spontaneous spike discharge. which partly explains the increased tension in nitrate solution. In experiments in which no change in spike frequency was observed, the increased tension might still have been secondary to a change of spike duration and configuration. However, indirect evidence indicated that nitrate might have an effect on tension apart from its effect on the electrical activity. Hodgkin and Horowitz[1] found that the K concentration required to produce a given tension in isolated striated muscle fibres was greatly reduced in nitrate solution. At 37°C the taenia coli maintained tension only as long as spike discharge persisted and usually it did not respond with a contracture to a depolarization with high KCl. Exposure to 10–20 times the normal concentration of KCl caused depolarization, stopped spikes and the muscle relaxed. When all the NaCl has been replaced by KCl, replacement of KCl with KNO_3 caused repolarization and initiated spikes, in spite of the 27 times the normal K concentration. However, in solution in which solid KCl was added or NaCl was only partly substituted, replacement of NaCl with $NaNO_3$ caused a slow increase in tension while the muscle remained depolarized and no spikes were recorded. It is therefore likely that nitrate has an effect on tension which is not mediated by its effect on the electrical activity. All experiments were done at 37°C. Considerable difference in the contractile response of taenia coli has been observed at lower temperature.

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515 Electrical Activity in Nerve-Free Smooth Muscle. A. W. Cuthbert (United Kingdom).

The smooth muscle of the chick amnion is devoid of nervous elements. It is useful to study, in this preparation, the direct effect of drugs on smooth muscle uncomplicated by effects on nerves or ganglia.[1]

Strips of chick amnion were examined in the sucrose gap apparatus,[2] one end of the preparation being connected to a mechano-electrical transducer. Simultaneous records of membrane potential and tension were obtained photographically. Most preparations exhibited spontaneous rhythm, showing slow action potentials just preceding small increments in tension. The action potentials showed small prepotentials and were followed by a phase of hyperpolarization, characteristic of smooth muscle.[3] Action potentials varying between 1 and 12 mV were seen at frequencies of from 1 to 6 per min. They were of long duration (3–5 sec) and resembled action potentials from the stomach[4]; sometimes an initial fast spike was followed by a plateau of depolarization. The value of the membrane potential, obtained by depolarization with isotonic K_2SO_4, was about 30 mV in 10 day membranes.

Acetylcholine is present in the chick amnion[5] and when given at a concentration of 10^{-6} g/ml, it caused an increased rate of firing of action potentials with a rise in the membrane potential of a few mV.

The response to rapid stretch was a burst of 3 or 4 action potentials with concomitant increases in tension. The effects of drugs on membrane potential and tension of the chick amnion is under study.

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516 The Role of Calcium in the Effect of Acetylcholine and Adrenaline on Smooth Muscle. K. A. P. Edman and H. O. Schild (United Kingdom).

Smooth muscle which has been depolarized by immersion in isotonic K_2SO_4-Ringer still responds to acetylcholine (ACh) and adrenaline.[1] In the

depolarized as well as in the polarized smooth muscle the presence of Ca is found to be essential for the initiation of contraction. The contractile responses of ACh and the α-effect of adrenaline show a very similar Ca dependence. Thus, in the depolarized rat seminal vesicle, the isometric responses to ACh and adrenaline decline at the same rate when Ca is removed from the bathing fluid and are restored at the same rate when Ca is reintroduced.

Calcium itself induces a slow sustained contraction of the depolarized smooth muscle.[2] However, ACh and adrenaline added on top of a Ca contraction produce a rapid further increase in tension, even in a preparation maximally contracted to Ca.

The contractile effect of Ca is strongly antagonized by the β-effect of adrenaline. In the depolarized rat uterus a substantial inhibition of the Ca-induced contraction is produced by 10^{-9} adrenaline and *iso*prenaline.

The experiments can be fitted into the hypothesis that Ca is an early link in the initiation of muscular contraction. ACh and the α-effect of adrenaline, although obviously acting on different receptors since they are antagonized by different antagonists, may have a later reaction step in common, a mobilizing of intracellular Ca.

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517 Sodium Exchange in Smooth Muscle. P. J. Goodford (United Kingdom).

It has been shown that a steady state was reached when pieces of guinea-pig taenia coli were set up in oxygenated Krebs' solution at 35°C, and that the Na content, K content and inulin space of the smooth muscle became constant.[1] Nearly all the Na in the tissue exchanged with a half-life of 0·5 min when the bathing solution had been labelled with ^{24}Na, and this exchange was too rapid to permit any resolution into intracellular and extracellular components.

Other pieces of taenia were equilibrated in a normal Ringer's solution and then transferred to one in which all the Na salts had been replaced by Li; the Na again washed out of the tissue with an initial half-life of 0·5 min but a portion remained in the muscle during the one hour immersion in the Li Ringer. As it was found[2] that the Na content of the taenia approached that of the external bathing solution after equilibration of the muscle at 4°C, the experiment in Li solution was repeated at that temperature and the proportion of non-exchangeable Na was then much greater.

It is suggested that the Na which remained in the tissue in the presence of Li was intracellular. The low content of bound Na at 35°C would then indicate a low intracellular concentration, which, however, approached the concentration in the external solution at 4°C. This observation may be used to interpret the normally very rapid Na exchange, and to resolve its separate components.

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518 Action of Cardiac Glycosides on a Smooth Muscle. T. Godfraind and A. Godfraind De Becker (Congo).

A piece of guinea-pig ileum was used and bathed by an oxygenated Tyrode solution. Contractions were recorded with an isotonic frontal lever. Several cardiotonics have been studied (digitoxin, ouabain, k-strophanthoside, 5×10^{-8} to 5×10^{-4} v/wt). All cause a contraction of the muscle; relaxation appears in the same solution. There is no relation between the dose and the height of contraction. This is a tachyphylactic action.

The action of these drugs on cholinergic or histaminergic muscle stimulation appears to be biphasic. First we observed a potentiation of the effects of acetylcholine and histamine. This potentiation occurs simultaneously with the contractile response of the ileum to the glycoside and appears only when contraction is produced by chemical transmitters. When the intestine is stimulated coaxially, the phenomenon still appears but in the presence of atropine, no potentiation is evoked.

This first phase is followed by a competitive inhibition of the effects of agonists. Several factors are concerned with this inhibitory action: the concentration of glycoside in the bath, the time of contact before contraction and the contractile activity of the muscle itself. Graphical representation of these factors give a typical saturation curve. There is an obvious parallelism between these data and the influence of the same factors on ion exchanges. Another point of similarity is the action of desoxycorticosterone. This drug which depresses the effects of glycosides on ion movements, reduces also their competitive action against acetylcholine and histamine.

519 Effect of Hydrochlorothiazide on Renal Electrolyte Gradient in Glucose Diuresis and Diabetes Insipidus. J. E. Baer, A. V. Brooks and R. M. Noll (U.S.A.).

In edematous patients, hydrochlorothiazide produces saluresis and extensive water diuresis. In diabetes insipidus, on the other hand, thiazides actually diminish urine flow while rendering the urine more hypertonic. The present experiments were designed to relate these actions of hydrochlorothiazide to the magnitude of the electrolyte gradient in the kidney.

Hydrochlorothiazide (100 mg/kg/I.V.) did not affect the renal electrolyte gradient in uninfused

dogs. The infusion of 5 per cent glucose at 10 ml/min alone obliterated the renal electrolyte gradient that is present in normal uninfused dogs. Hydrochlorothiazide did not restore the gradient in the glucose-infused dogs.

In rats with experimental diabetes, a marked gradient occurred, the sodium content increasing from 70 meq/kg wet wt. in the cortex to 500 meq/kg wet wt. in the papilla. Administration of hydrochlorothiazide for 3 days (45 mg/kg orally in divided doses) decreased urine flow and water intake promptly: electrolyte excretion decreased more slowly. The sodium gradient was minimally affected by the hydrochlorothiazide treatment.

520 The Role of Hyaluronidase in the Action of Antidiuretic Hormone. A. G. Ginetzinsky (U.S.S.R.).

In the state of dehydration the urine of all terrestrial vertebrates contains hyaluronidase. Enzymatic activity of the urine drops to the zero level after water loading when the secretion of ADH is inhibited. When suitable experimental conditions excluding electrolyte and dilution effect are created the correlation between hyaluronidase activity of the urine and the extent of diuresis is maintained.

Hyaluronidase inhibitors when administrated directly into the renal artery increase diuresis. This effect appears on the side of the injection only.

If the structures producing antidiuretic hormone are destroyed hyaluronidase disappears from the urine, but after pitressin injection the urine reacquires fermentative activity.

By means of the stop flow method, it was found that hyaluronidase appears in the urine produced by the distal parts of the nephron. Proximal urine contains no enzyme. Cytological and histochemical investigations showed that renal epithelium acted upon by ADH secretes a substance containing protein.

The intercellular cement and the basement membrane of the collecting ducts and of the distal parts of the nephron contain hyaluronic acid. The ADH effect is produced by means of hyaluronidase liberation. The enzyme depolymerizes hyaluronic acid which makes the tubule wall waterproof. Accordingly, its permeability increases. The osmotic free water then is reabsorbed and antidiuresis occurs.

521 Renal Transport of Organic Anions: A Unifying Hypothesis. A. Despopoulos (U.S.A.).

Active renal tubular excretion of organic anions has been examined in an attempt to define the nature of the physico-chemical interaction between transported substrates and a postulated receptor in renal cells. Comparison of more than 150 organic anions permits tentative description of a general molecular structure which defines substrate specificity of the transport system. Intramolecular components which appear to be essential for transport include an unsaturated oxygen group (—C:O; —S:O) and an ionic function (—COOH; $-SO_3OH$; $-SO_2NH-$). A three-point interaction with the receptor can be satified by formation of a hydrogen bond at the unsaturated oxygen (—C:O; —S:O) and both a hydrogen bond and an ionic bond at the anionic group. These postulates are invoked to account for participation of such widely divergent structures as hippurates, penicillins, sulphonates, sulphonamides and organomercurial diuretics in what appears to be a single transport function.

522 The Effect of Diuresis and Diuretics upon the Renal Tubular Transport of Alkaline Earth Cations. M. Walser and J. R. Trounce (U.S.A.).

Recent studies in this laboratory indicate that the major determinant of the renal clearances of Ca and Mg in the dog is the simultaneous clearance of Na. Salt-restricted animals excrete little Ca or Mg, even during mannitol diuresis. Infusion of saline leads to an increase in the clearance of Ca (corrected for protein-binding) which is approximately equal to the change in Na clearance; Mg clearance increases even more.

These studies have been extended to an examination of the effects of diuretic drugs. Dogs were infused with saline at 5 ml/min until urine flow exceeded 3 ml/min. Following several control periods, either chlormerodrin, 4 mg/kg; chlorothiazide, 10 mg/kg; or hydrochlorothiazide, 5 mg/kg were injected intravenously. Chlormerodrin led to increases in Ca and Mg clearance even greater than the increases in Na clearance. The thiazide derivatives decreased alkaline earth excretion although the natriuretic responses were comparable to those obtained with chlormerodrin. Changes in filtration rate or urine pH were minor and could not account for these results.

The effect of ouabain was studied by injecting 50 μg/kg into one renal artery. Ca and Na clearances rose in parallel several fold, in the injected kidney but not in the contralateral kidney. Filtration rate either fell or remained constant.

The drugs studied evidently exert profound and distinct effects upon the renal tubular transport of alkaline earth cations in addition to their known actions upon Na reabsorption.

523 Antidiuretic Responses to Neurohypophysial Hormones and Some of their Synthetic Analogues in Dogs and Rats. W. H. Sawyer, W. Y. Chan and H. B. van Dyke (U.S.A.).

The antidiuretic and vasopressor potencies of neurohypophysial peptides are not necessarily proportional. The relative antidiuretic activity of a peptide may also depend on the species in which it is tested. By means of intravenous injection of

several neurohypophysial hormones and synthetic analogues in dogs and rats we have studied the relations between molecular structure and antidiuretic responses. USP Standard, containing arginine vasopressin, was used as the basis for all comparisons.

Peptides with lysine in the 8-position (lysine vasopressin, lysine vasotocin, 2-phenylalanine lysine vasopressin), when compared to 8-arginine peptides (arginine vasopressin, arginine vasotocin), have less antidiuretic activity in dogs than in rats. 8-leucine analogues (oxytocin, oxypressin) are as active in dogs as rats. 8-arginine peptides produce a protracted antidiuresis in rats while antidiuretic responses to 8-leucine analogues are briefer, and those to 8-lysine analogues are even more transient. There are no evident relations between molecular structures and the duration of antidiuretic responses in dogs or of vasopressor responses in rats. The amino-acid in the 8-position of a peptide appears to be important not only to relative antidiuretic potencies in dogs and rats but also to the duration of antidiuretic action in rats.

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524 Histochemical Demonstration of Mercury in Renal Cells. E. J. Capruny (U.S.A.).

Mercurial diuretics act directly on the kidney to inhibit transport of NaCl and K. Dimercaprol (BAL) re-establishes transport by removing Hg from the kidney. Evidently the metal must occupy receptors in order to retard electrolyte transport. A method for localizing Hg in renal tissue should, therefore, afford significant information pertaining to the cellular sites and mechanism of action of mercurial diuretics. A method and preliminary results are tendered in this report. All studies were performed using mongrel dogs and albino rats. Animals were treated with 1–5 mg Hg/kg body wt. either as one of 7 organic compounds or inorganic $HgCl_2$. Portions of kidney were fixed in acetone and 10 per cent trichloroacetic acid (TCA). Slices were stained for Hg using dithizone dissolved in *N,N*-dimethylformamide. Tissues fixed in TCA were embedded in paraffin, cut on a microtome (10–20 μ), and stained. Colour developed only in the renal cortex of tissue slices and was most intense in kidneys of animals treated with $HgCl_2$. Distribution patterns were examined in histological sections prepared from dogs treated with mercuric cysteine. Hg was discernible in cytoplasm but not in nuclei of proximal and distal tubular cells. Basal portions of the cells appeared to contain larger concentrations. Hg was not apparent in tubular lumens, glomerular epithelium, or interstitial tissue. Presence of the coloured Hg-dithizone complex was verified by microphotometric analysis. Light absorption in cells was found to be maximal at 485 mμ, which is also the peak absorption band of the complex dissolved in chloroform.

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525 Sodium Transport in Rats with Aminonucleoside Nephrosis. G. Senft (Germany).

Oedema formation in rats with aminonucleoside nephrosis is accompanied by a decrease in glomercular filtration rate and by an increase in the percentage of the glomerular filtrated sodium reabsorbed by the kidney tubules.

The experiments reported in this paper were undertaken in order to find out if the relative increase in the amount of sodium reabsorbed is caused only by a decreased glomerular filtrate almost completely reabsorbed by a normally functioning transport system. This can be explained by the prolonged contact of the urinary sodium with tubular cells.

In addition, evidence has been obtained that aminonucleoside induces augmentation of the reabsorptive capacity of the kidney tubules.

These results were supported by experiments in which the distribution of sodium-ions between extrarenal intra- and extra-cellular compartments has been measured.

526 Influence of a Nephrotoxic Agent on the Renal Concentrating Mechanism in the Rat. G. Peters and P. R. Hedwall (Germany).

Aristolochic acid (AA) is a nephrotoxic agent[1, 2] said to abolish vasopressin antidiuresis in rabbits.[2] Its effects on the kidney function of rats were investigated. A single dose (30 mg/kg i.v.) causes polyuria reaching a peak on the 8th day after injection. Water excretion during thirst periods was increased 4-fold without a corresponding increase in electrolyte excretion. Histological studies showed mild degenerative changes in proximal as well as distal tubuli, while glomeruli and collecting ducts appeared undamaged. The renal concentrating ability was greatly depressed on the 8th day after injection. Maximum urinary osmolarities were 995 ± 81 mosm/L (normal controls with comparable urinary osmotic loads: 2092 ± 103 mosm/L). The contribution $(Na^+) + (K^+)$ to total osmolarity was reduced from 0·19 ± 0·01 to 0·16 ± 0·01. Exogenous vasopressin (100 mU/kg s.c.) injected during water diuresis, increased urinary osmolarity from hypotonicity to 549 ± 68 mosm/L in AA treated rats and to 631 ± 56 mosm/L in controls. The contribution of electrolytes to the increased osmolarity was depressed in the AA-treated rats. GFR (Cl_{In}) was depressed to 1/6, Cl_{urea} to 1/2 its normal value. The clearance ratio, $Cl_{urea}/Cl_{Endag.\ Cr.}$, was increased from 0·67 to unity. It would, therefore, appear that the passage of urea from tubular fluid to interstitial fluid and blood was impaired after treatment with AA. This passage has been shown to contribute to the concentrating mechanism,[3] and to be enhanced by vasopressin in dogs.[4] The decrease in "permeability" to urea caused by AA or other agents may

be a factor in limiting renal concentrating ability in addition to other known factors.[5, 6, 7, 8, 9]

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527 Accumulation of ^{35}S labelled Probenecid in Guinea Pig Kidney Slices. W. Braun and H. Schniewind (Germany).

The excretion of actively transported organic acids across the renal tubular cells is inhibited by probenecid. The mode of action of this effect is not understood as yet. Beyer has shown that the parent compound *p*-carboxybenzenesulphonamide itself is secreted.

To study this problem further, experiments have been performed with the aid of ^{35}S labelled probenecid. It was found that probenecid was highly concentrated in rat kidneys following a single injection. Relative concentrations in kidney, liver, heart and skeletal muscle were 100, 27, 12 and 2 respectively. Furthermore, accumulation of probenecid in kidney slices has been investigated by the method of Cross and Taggart and Beyer *et al.* Probenecid is readily taken up and highly concentrated by guinea-pig kidney slices. Plotting the substrate concentration against accumulated amount yields a saturation type curve, the Michaelis constant of which is calculated to be $4 \cdot 10^{-5}$ M.

From these results we may conclude that probenecid is also actively transported by the renal tubules. Its affinity to the carrier system however is about 15 times as great as that of PAH. Probenecid therefore seems very likely to act as a competitive inhibitor like bromcresolgreen and bromphenolblue with high affinities and low turnover, though the Lineweaver–Burk plot shows the characteristics of a non-competitive inhibition.

528 Osteoarthritis Treated with a Placental Extract. A Double Blind Study. L. W. Granirer (U.S.A.).

Impressed by the rarity of osteoarthritis during pregnancy, the author investigated the effect of a placental extract in patients with this disease.

This is a controlled study of the effects of a placental extract in osteoarthritis. Eighty-four patients with osteoarthritis were studied in a double blindfold manner: one half received a placebo and the other half received the placental extract. The placental extract was a crystalline preparation which was injected subcutaneously in doses of 150 mg once or twice weekly. The cases were followed for one year.

Moderate to excellent subjective and objective improvement was observed in 91 per cent of the placental treated patients as opposed to 15 per cent of the control group.

There were no toxic manifestations, no evidence of sensitivity or diminishing response to therapy in the placental-treated group. Their functional capacity as well as their sense of well being was improved. The incidence of destructive bone lesions by roentgen examination was reduced and the improvement was still evident to a significant extent at the end of the year.

From the results, the author believes that osteoarthritis is a deficiency disease caused by an inborn error of metabolism and that this deficiency may be corrected by replacement therapy with the placental extract which probably controls the release of essential hormones.

Excellent results can be obtained in many patients by the relief of joint pain and swelling, often with long-term beneficial effects.

529 On the Pharmacology of Reactivity (Experiments with Ginseng). V. Petkov (Bulgaria).

Pharmacological substances should be considered and studied not only as factors which can change the functions of the organism in one direction or another but also as factors which, without even provoking directly manifested effects, can change the reactivity of structures of the organism in such a manner that the subsequent (or simultaneous) influence of most varied factors (including also pharmacological ones) would result in a reaction different from that usually expected. On the other hand, one would think that by means of such pharmacological substances we should also be able to influence, in a direction we desire, the pathologically changed reactivity in morbid conditions.

In experiments with ginseng it has been established that extracts from the roots of this plant facilitate the formation of conditioned reflexes of signal stimuli and make possible the manifestation of the effect of subthreshold doses of phenamine stimulating the conditioned reflex activity. At the same time, EEG investigations show that ginseng facilitates the occurrence of primary evoked potentials. In experiments on a straight abdominal muscle of a frog, in a combined application of ginseng and acetylcholine, a potentiation of the effects is observed. In threshold concentrations, ginseng

and acetylcholine mutually potentiate their stimulating action on the intestinal motility. The results obtained from further experiments with pendiomide, glutaminic acid, eserine, tubocurarine, $CdCl_2$, KCl, atropine, $BaCl_2$ and histamine suggest that the influence of ginseng on the reactivity of the nervous and muscular elements is primarily due to its influence on the intracellular potassium storage.

The effects of applying ginseng may only partially be explained as the result of its direct stimulating action. The operation of ginseng should be assessed much more as a consequence of its influence on the readiness of the nervous and muscular and perhaps of other physiological systems to react to some or other stimuli. An attempt is being made to substantiate a new pharmacology of reactivity.

530 Hormonal Effects of Different Plants. A. Sharaf and I. Akkad (U.A.R.).

When the oestrogenic, androgenic, progestational and gonadotrophic effects were examined, it was found that different plant preparations possess some of these hormonal properties. The sweet orange juice and ascorbic acid produced endometrial progestational proliferative reactive. In this respect, it possesses a progesterone-like activity and may be used alone or in combination with progesterone in all conditions associated with low level of progesterone as in habitual and threatened abortions. *Cicer arientum*, has been experimentally proved to produce androgenic activity as it increased the weights of the prostates and seminal vesicles of castrated male rats, and progesterone-like action as it produced progestational proliferation in the endometrium of ovariectomized rabbits. *Catha edule* leaves, when fed to animals, also produced progestational prliferation in the endometrium of rabbits resembling that of progesterone. On feeding Egyptian clover to immature rabbits it increased the uterine weights to a considerable extent; this indicates that clover possesses oestrogenic activity.

The oxytocic effect of different plant preparations has been investigated *in vitro*, on the uterus of animals at various stages of the sex cycle and after hormonal treatments. Quinine produced strong stimulation of the uterine muscle of pregnant rats and ovariectomized animals treated with progesterone. The effect was less marked on the uterus of oestrous rats and of overiectomized animals treated with oestradiol. Quinine also increased the uterine response of the pregnant uterus to the posterior pituitary extract. Cotton root bark extract increased the uterine motility of the pregnant uterus and that of ovariectomized animals treated with progesterone, whereas, it inhibited that of oestrous and ovariectomized rats treated with oestradiol. On the other hand, *Lupinus termis* seed extract produced consistent motor effects on the uterus of all animals whatever the state of the uterus.

531 Mode of Action of Nigellone. M. Mahfouz and M. El-Dakhakhiny (U.A.R.).

Nigellone is the active principle of the seed oil of *Nigella sativa L.*, which has recently been isolated in this department in a pure crystalline form and was shown to possess a molecular formula of $C_{16}H_{22}O_6$. The most prominent pharmacological property of Nigellone was its power to protect guinea pigs against histamine-induced bronchospasm, a property present in the mother oil to a very small degree. It also proved to be a valuable therapeutic aid in the control of bronchial asthma in children and adults. Administered as oral drops it was used in the treatment of over 200 cases with favourable improvement in about 70 per cent of cases. Nigellone had no toxic effects even when given in large doses to men or in animal experiments.

The possible mechanism of this action was investigated. Stimulation of the adrenergic mechanism could not be demonstrated especially if other pharmacological actions of the drug are considered, e.g. no vasoconstrictor effect on perfused blood vessels, nor any hypertensive effect on experimental animals or man. There was also no evidence that Nigellone had a blocking action on the peripheral cholinergic mechanism as it did not antagonize the effects of acetylcholine on the bronchi, intestines, etc.

The available evidence points to the fact that the drug is transformed to an active form in the blood which inhibits the dynamic activity of the plain muscle fusiform cell membrane.

532 Antitussive Action of Oxolamine (3-Phenyl-5-β-Diethylaminoethyl-1:2:4-Oxadiazole). B. Silvestrini (Italy).

Oxolamine (3-phenyl-5-β-diethylaminoethyl-1:2:4-oxadiazole), a new compound synthetized and studied in the Angelini Research Laboratories, presents the following properties:

(a) an antitussive action
(b) an analgesic–anti-inflammatory action of the aspirin-type
(c) a spasmolytic action of the papaverine-type
(d) a mild antipyretic and local anaesthetic action.

The antitussive action of oxolamine is particularly evident against experimental cough induced by irritating vapours, which produce inflammation and spasm of the bronchial tree. Oxolamine is less active against centrally evoked cough. The possibility is therefore discussed that the antitussive action of oxolamine is taking place through a synergism between the mild depressive action on the coughing centre and the anti-inflammatory and spasmolytic properties.

533 The Behaviour of Hypertensive Drugs on the Suprarenal Gland and Kidney Perfusion in the Cat. P. D. Garcia de Jalon and L. A. L. Santos (Spain).

The pharmacological controls of 5HT were

performed by the Vane method and those of adrenaline and hypertensin on the isolated rat uterus and the determination of pitressin on the blood pressure of pithed rats.

From the hypertensive drugs used, only adrenaline revealed a marked vasoconstrictor effect which was abolished by adrenolytics such as Duvalidan or Ilidar.

Doses of 100–200 μg of 5HT, of 200–500 μg of hypertensin and of 100–200 mU of pitressin produced little vasoconstrictor effect in the perfused kidney, and decreased the diuresis very little; only during perfusion of both the suprarenal gland and the kidney could slightly greater effects be obtained.

We suggest that these results may be due to a greater sensibility of the suprarenal tissue to such drugs; we do not believe in a possible release of adrenaline since we could not demonstrate this using the "isolated rat uterus". The previous administration, 24 hr before, of 25 or 50 mg of Iproniazid did not increase the effects of 5HT. On the other hand, 5HT does not seem to be stored in the blood cells, since the values of 5HT in hemolysed samples were not greater than those of the blood plasma.

Samples of blood plasma obtained 90 sec after the administration of pitressin and hypertensin did not give any blood pressure responses, nor did they influence the rat uterus.

There is a possibility that the small effect upon the kidney of vasoactive drugs may be due to heparinization; O'Connor, Verney, Vogt (1940) stated that the vasoconstrictor factor was not produced using heparinized blood. Some enzymatical renal factor might be responsible for the inactivation of 5HT, hypertensin and pitressin in the presence of heparin; possibly by a peculiar enzymatical destruction of the drugs or the existence of a renal pool.

534 The Actions of Hepatocatalase upon Cholesterol and on Other Metabolites. J. Laporte, P. P. Muset and F. G. Valdecasas (Spain).

Besides the specific properties which are already known, hepatocatalase acts by inhibiting the formation of lipoperoxides *in vitro* by the enzyme lipoxidase. In addition, we have observed other antagonisms between the enzymes lipoxidase and hepatocatalase in animals.

In these studies, we have been able to establish an action by hepatocatalase on certain metabolic processes. There is a diminution of the increase in creatine, uric acid and 17-hydroxy-corticosteroids, etc. induced by the administration of lipoxidase or by other mechanisms and, clinically, an improvement occurs in various pathological conditions following prolonged administration of hepatocatalase. The normalizing action on increased levels of cholesterol is specially interesting. Other actions have also been found: (1) facilitating the survival of animals with anoxia; (2) potentiation of some analgesics; (3) decrease in the toxicity of digitalis; and (4) decrease in the cachectizing effects effects of medium doses of X-rays.

The systematic use of this enzyme has been made possible by using a method devised by us which stabilizes it in permanent solid form. This consists of lyophilizing the cystalline enzyme in a solution of disaccharide.

Although the enzyme lacks acute and chronic toxicity even at doses higher than 10,000 U/kg, the pharmacologically effective dose is limited as hepatocatalase may produce no effect either in large or small doses.

535 Isolation and Purification of an Anti-hypertensinic, Hypotensive Enzyme of the Soya Bean. F. G. Valdecases, J. Martin, P. Muset and J. Laporte (Spain).

Defatted soya bean meal is extracted with acetate buffer at pH 4·5 and the extract (after treatment with barium acetate, acetone and lead acetate), is fractionally precipitated with ammonium sulphate and the active fraction is subjected to a partial thermic denaturlization at 60°C. Finally, the product obtained is chromatographied in equilibrated DEAE cellulose colums at pH 7; the elution is carried out with sodium chloride solutions of increasing concentrations from 0·02–0·3 M. During this last process a series of proteins with interesting biochemical and pharmacological activities are obtained, and among them an enzyme of great antihypertensinic activity.

When this enzyme is incubated with hypertensin, the amino-acids of this octapeptide are liberated.

Further study of its enzymatic properties and characteristics by testing it with natural and synthetic substrates as well as in the presence of several substances which commonly act as inhibitors or activators, seems to show that is a proteolytic enzyme of unusual characteristics which do not conform well with any of those described up to the present time.

536 Pharmacological Actions of the Lipoxidase Enzyme and its Isolation by means of a New Technique. P. Muset, J. Martin, J. Laporte and F. G. Valdecases (Spain).

In order to study the peroxidative processes in the animal organism, the lipoxidase enzyme has been used. It has been extracted from the soya bean by ourselves and from other samples from other laboratories.

Injection of this enzyme in animals (rats, guinea pigs, cats and dogs), produces a variety of reactions, among others specially drowsiness ,paralysis, pulmonary exudation and, later on, hair loss. Other actions of a metabolic type, are increase excretion of uric acid, creatine, 17-hydroxycorticosteroids, etc.

Its action is parallel and additive to that pro-

duced by roentgen radiation, with which it has in common peroxide formation.

Detailed study of some other properties, has shown that in the lipoxidase which has been used there were other protein fractions without lipoxidase activity.

For the isolation of pure lipoxidase in quantity, the technique of Theorell has been modified, using chromatography in a column of DEAE cellulose and elution with solutions of different molarities of sodium chloride. By this method a fraction of lipoxidase in electrophoretically pure condition has been obtained. Other fractions with other enzymatic activities have been separated.

The formation of lipoperoxides *in vitro* by lipoxidase is inhibited by the presence of hepatocatalase in the medium. In the living animal the actions of lipoxidase may be totally or partially antagonized by means of the hepatocatalase enzyme.

537 An Anti-hypertensinic Enzyme; its Action on Blood Pressure. F. G. Valdecasas, J. Laporte, P. Muset and J. Martin (Spain).

During the investigation of several protein fractions of the soya bean and specially of the "lipoxidasic fraction" of Theorell, we observed an hypotensive action of unusual characteristics: i.e. retarded effects even after intravenous injection and a prolonged duration of action which does not increase proportionally with augmentation of the dose above a certain limit. All this suggests that there exists an indirect effect mediated by some physiological substances.

By using chromatographic techniques at low temperature, we have succeeded in isolating a protein which, besides its hypotensive action, appears to be a proteolytic enzyme of great selective activity upon the peptide hypertensin. Concentrations of 0·05 mg/ml inhibit the activity of a hypertensin solution containing 20 mg/ml in 30–60 min.

Further, its proteolytic action upon other peptides (ACHT, oxytocin and vasopressin), protamins and proteins, has been investigated. It only shows activity on denatured haemoglobin, salamin and cuplein. It does not alter the coagulation time of the blood and shows no proteolytic action upon casein. It is not sensitive to iodoacetate, phenantrolin and *p*-mercuric-chloro benzoate. It does not keep well in solution at room temperature and is rapidly destroyed at 60°C. When lyophilized it is perfectly stable.

A method of evaluation, based on the destruction of hypertensin, has been developed: the enzyme is incubated with Hypertensin Ciba at 25°C, for 60 min and afterwards the hypertensin is evaluated by the cat blood pressure test. The anti-hypertensinic unit is defined.

The enzyme shows very little toxicity and is not anaphylactogenic.

538a Thiamine et Dithiopropylthiamine. Etude de leur Métabolisme chez la Souris et chez le Rat, par marquage au ^{35}S. Y. Cohen, A. Uzan et G. Valette (France).

La dithiopropylthiamine est un dérivé liposoluble de la thiamine qui conserve les propriétés antinévritiques de la vitamine B_1 et qui, par voie orale se montre plus actif.

Nous avons recherché la cause de cette différence d'activité par l'étude comparative du métabolisme de la thiamine ^{35}S et de la dithiopropylthiamine ^{35}S administrées par voie orale chez la souris at chez le rat. Les méthodes d'autoradiographie, de mesure de la radioactivité, de chromatographie nous ont conduits aux résultats suivants.

(1) Chez la souris: Il n'y a pas de différence qualitative dans la distribution de la radioactivité selon qu'on administre l'un ou l'autre produit. Quelques organes se chargent préférentiellement: le foie et le rein qui sont fortement actifs, le myocarde, les graisses interscapulaires et le cerveau qui retiennent la radioactivité de façon moindre.

(2) Chez le rat: La mesure de la radioactivité des principaux organes (foie, rein, coeur, cerveau) montre une concentration constamment plus élevée pour la dithiopropylthiamine que pour la thiamine. Dans le foie la radioactivité décroit dans le temps; dans le rein elle demeure basse; au contraire dans le cerveau et dans le myocarde elle croit progressivement dans les 24 heures. L'excrétion urinaire est plus importante pour la dithiopropylthiamine que pour la vitamine B_1 par suite d'une meilleure absorption intestinale du dérivé dithiopropyle.

L'analyse chromatographique montre que dans le foie la dithiopropylthiamine est transformée en thiamine.

En conclusion. La comparaison du métabolisme de la thiamine et de la dithiopropylthiamine, antinévritiques, nous permet de penser que la différence a leurs propriétés biologiques est essentiellement d'ordre quantitatif.

538b Thiamine and Dithiopropylthiamine. A Study of their Metabolism in the Mouse and Rat by Means of Labelling with ^{35}S. Y. Cohen, A. Uzan and G. Valette (France).

Dithiopropylthiamine is a fat-soluble thiamine-derivative which retains the antineuritic properties of vitamin B_1 while being more active when administered by mouth.

The cause of this difference in activity has been investigated by a comparative study of the metabolism of thiamine ^{35}S and of dithiopropylthiamine-^{35}S administered to mice and rats by mouth. The methods of autoradiography, of measurements of radioactivity and of chromatography have shown the following results.

(1) In the mouse: There is no qualitative difference between the distribution of radioactivity following

the administration of these two substances. Certain organs become selectively charged: the liver and kidney were very active; the myocardium, intrascapular fat and the brain retained radioactivity to a lesser extent.

(2) In the rat: Measurements of the radioactivity of the principal organs (liver, kidney, heart, brain) showed a constantly higher concentration of dithiopropylthiamine than of thiamine. In the liver the radioactivity decreased with time; in the kidney it remained low; in the brain and myocardium, however, it increased progressively during the course of 24 hr. Excretion in the urine affected dithiopropylthiamine more than vitamin B_1, as dithiopropylthiamine is absorbed to a greater extent by the intestine.

Chromatographic analysis shows that dithiopropylthiamine is transformed to thiamine in the liver.

Conclusion: Comparison of the metabolism of the antineuritics thiamine and dithiopropylthiamine leads to the conclusion that the difference in their biological properties is essentially quantitative.

539 Thiamine as a Nicotine Antagonist. I. YAMAMOTO (Japan).

In our serial study on nicotine metabolism and its detoxication, thiamine was proved to be antagonistic to nicotine in various organs *in vitro* and this antagonism was stronger than that of acetylcholine or histamine suggesting that one of principal sites of action of thiamine is located in nerve ganglion. From activity–concentration curves, this antagonism appeared to be a so-called competitive one being more typical than those of hexamethonium and TEA. Thiamine is vasodepressing in dogs and not associated with adrenergic action but antagonistic to the pressor effect of nicotine and bilateral occlusions of the common carotid. It inhibits the depressor response to vagus stimulation. These findings and those obtained from experiments on cat nictitating membrane may be taken as illustrations of a ganglionic blocking effect of thiamine. Thiamine displayed a supressing effect probably based on the blocked adrenaline release of nicotine, on the nicotine-induced hyperglucaemia in rabbits.

Diuresis by cigarette smoking was significantly inhibited by thiamine in man while it exerted no influence on the diuresis induced by hypophyseal posterior lobe extract suggesting that nicotine antagonism by thiamine is connected with the hypothalamo-posterior-pituitary system and, furthermore, it is interesting to note that no other nicotinolytic substances showed this type of antagonism.

Among related compounds to thiamine, thiazole derivatives containing an amino group at "2" and/or phenyl group at other than "2" elicited an augmentation in antagonistic activity to nicotine.

540 Non-Hydrazide Monoamine Oxidase Inhibitors and Their Effects on Central Amines and Motor Behaviour. G. M. EVERETT and R. G. WIEGAND (U.S.A.).

Two series of non-hydrazide MAO inhibitors have been investigated for pharmacologic responses and biochemical changes. Levels of DOPA, dopamine, norepinephrine, epinephrine and serotonin were determined in whole mouse brain after giving MAO inhibitors alone or in combination with DOPA.

MO-911 (A19120) (N-benzyl-N-methyl-2-propynylamine hydrochloride) is an active MAO inhibitor *in vitro* and *in vivo*. The central amines gradually increase concomitantly with increasing motor activity and aggressiveness. These effects are enhanced after giving DOPA.

MO-1255 (A19757) (ethyl-N-benzyl-N-cyclopropylcarbamate) appears inactive by *in vitro* tests, but is a potent MAO inhibitor *in vivo*. Its effect on central amines are similar to MO-911. Both MO-911 and MO-1255 are five times more potent than iproniazid as determined by the *in vivo* DOPA response test in mice. MO-1255 has a duration of only 3 days as compared to 8 days for iproniazid or MO-911 in the mouse. Neither of these new drugs is a direct central stimulant. Nevertheless, after giving the drugs to dogs, cats and monkeys, gradually increasing motor activity and irritability develops over a period of a day or more and then slowly subsides.

The parallel data on central amines and behavioural response suggest a close correlation between dopamine and norepinephrine levels and increased motor activity and agressiveness.

541 Inhibition of Dopamine β-Oxidase by Amphetamine and p-Hydroxyamphetamine. M. GOLDSTEIN (U.S.A.).

Among several structural analogues of dopamine, amphetamine (phenylpropylamine) and *p*-hydroxyamphetamine (*p*-hydroxyphenylpropylamine) were tested as possible inhibitors of dopamine β-oxidase. The enzyme dopamine β-oxidase which catalyses the conversion of dopamine to norepinephrine was prepared from adrenal medulla by the method of Levin *et al.*[1] The relative inhibition rate of dopamine β-oxidase by amphetamine and *p*-hydroxyamphetamine was determined by comparing the amounts of norepinephrine-1-^{14}C formed in an incubation mixture which contained only the substrate (dopamine-1-^{14}C) and an incubation mixture which contained the substrate together with amphetamine or *p*-hydroxyamphetamine. The linear increase of inhibition with the amount of added amphetamine or *p*-hydroxyamphetamine suggests that these two compounds are in competition with the substrate for the same enzyme. It is likely that the enzyme (dopamine β-oxidase) which has been shown to be non-specific for dopamine[2]

also catalyses the oxidation of amphetamine and *p*-hydroxyamphetamine to the corresponding β-hydroxy compounds. Attempts are presently being made to isolate this compound. The correlation of the present findings with the pharmacological action of amphetamine and *p*-hydroxyamphetamine will be discussed.

1. Lewin, E. Y. *et al.* (1960), *J. Biol. Chem.*, **235,** 2080.
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542 Pharmacology and Biochemistry of α-Methyl-Dopa in Man and Experimental Animals. A. Sjoerdsma and S. Udenfriend (U.S.A.).

Of several compounds known to inhibit the decarboxylation of aromatic L-amino acids, α-methyl-3:4-dihydroxy-DL-phenylalanine (α-methyl-dopa) was selected for study in man. Levels of urinary amines were utilized as an index of decarboxylation, and patients with hypertension, malignant carcinoid, and phenylketonuria were treated with 1–6 g/day of α-methyl-dopa. This resulted in marked inhibition of the conversion of tyrosine, dopa, phenylalanine, tryptophan and 5-hydroxytryptophan to their corresponding urinary amines, confirming the *in vitro* demonstration of a single aromatic L-amino acid decarboxylase. During the experiments in man two pharmacologic effects were observed: (1) sedation, most marked initially but largely disappearing within 72 hr on continued therapy; and (2) lowering of blood pressure. The L-isomer of α-methyl-dopa was found to account for all biochemical and pharmacologic effects. Studies on 50 hypertensive patients for periods up to 15 months indicate considerable promise as an antihypertensive agent and possibly also as a tranquilizer.

Corollary studies in animals showed that single large doses of α-methyl-dopa cause depletion of serotonin in brain and nor-adrenaline in brain and heart. Surprisingly, the more marked depletion of tissue nor-adrenaline appears to involve release of the hormone and to be independent of decarboxylase inhibition and persists for 2–4 days after disappearance of enzyme block. By contrast the serotonin effect disappears within 15 hr. Current studies on this agent and other α-methyl-amino-acids in animals and patients should clarify the pharmacologic–biochemical interrelationships.

543 Further Studies on the Possible Rapid Synthesis of Norepinephrine from Administered 3:4:Hydroxyphenylethylamine. W. M. Booker and W. L. West (U.S.A.).

Previous experiments reported from our laboratory (1959 and 1960) have suggested the strong probability that dopamine administered to anaesthetized dogs may be synthesized rapidly (within 10 min) to norepinephrine. Adrenal vein and femoral artery plasma samples or their aluminium oxide extracts when tested on the isolate perfused guinea-pig heart preparations show increased inotropic responses over the control periods, indicating increases in catecholamines from the adrenal gland and possibly from other sources in the body. We have made further observations along the following lines which we are currently expanding in our experimental approach to this important problem: (1) Dopamine administered to adrenalectomized dogs (anaesthetized) results in increase in catecholamines in the femoral artery. This suggests that other sources (brain and sympathetic post-ganglionic nerve fibres) may be actively engaged in synthesizing catecholamines from dopamine; (2) Mice treated with dopamine and subsequently sacrificed, show increased quantities of norepinephrine in brain above the controls. These quantities of norepinephrine are even higher when the dopamine-treated animals are also treated with monamine oxidase inhibitors; (3) Reserpine, which sweeps out the catecholamine stores, may block the synthesis of norepinephrine, as reserpinized dogs, administered dopamine do not show a marked increase in catecholamines coming from the adrenal glands; (4) Repeated administration of acetylcholine to anaesthetized dogs and cats depletes the adrenal gland of its stores, but, when dopamine is administered, the catecholamines leaving the gland are increased within 10–20 min.

Data from a variety of experiments from each of the above areas will be presented.

544 Sympatholytic Drugs, DOPA Awakening Effect and Enzymes. T. L. Chruściel (Poland).

L-DOPA (L-3:4-dihydroxyphenylalanine) in large doeses produces an awakening effect in reserpine pretreated white mice and monkeys.[1, 2, 3] Guanethidine (Ismelin) increases this effect, whereas bretylium (Darenthin) does not change it. Phenoxybenzamine (Dibenyline) blocks the symptons of overactivity and excitement caused by L-DOPA in reserpine and iproniazid treated mice.[4] The results of the investigation of the influence *in vitro* and *in vivo* of the above sympatholytic drugs upon the activity of some enzymes (decarboxylase, serum and tissue mono-amine-oxidase) connected with the biogenesis and metabolism of catecholamines are reported and discussed.

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545 Evidence for a Central Mechanism in Angiotensin Induced Hypertension. J. P. Buckley and R. K. Bickerton (U.S.A.).

The purpose of this study was to determine if Angiotensin II possessed central hypertensive properties and, if so, determine the possible mechanism of action. Dog cross-circulation preparations, modified after that described by Taylor,[1] were utilized in this study. Leakage was determined using ^{131}I as the tracer substance and no leakage was observed over the 3-hr. experimental period. Angiotensin II administered into the arterial inflow to the recipient's head, in doses varying from 0·2 to 4 mcg/kg, produced consistent pressor responses in both the recipient and donor animals with pressor increments ranging between 12 per cent and 40 per cent above normal levels in the recipient and 30 per cent to 50 per cent above pre-drug level in the donor. Central hypertensive responses were shorter in duration (0·5 to 1·0 min) than the pressor responses in the donor animals (2·0 to 4·0 min). The central hypertensive response could be blocked by the administration of piperoxan 1·0 mg/kg into the recipient's peripheral circulation. The administration of Angiotensin II via the femoral vein still produced potent pressor responses. The data obtained in these studies suggest that Angiotensin II produced an increase in blood pressure via two mechanisms: (1) A central hypertensive effect, probably due to a stimulation of central sympathetic structures and evoking peripheral sympathetic discharges, which are blocked by the administration of sympatholytic agents into the peripheral circulation; (2) A direct peripheral action on the vascular smooth musculature.

(Supported by U.S.P.H.S. Grant H-3475 and Ciba Pharmaceutical Products, Inc.)

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546 Vasoconstrictor Effects of 5-Hydroxytryptamine and Related Substances. H. Weidmann and A. Cerletti (Switzerland).

With regard to vasoconstrictor activity, the sympathomimetic amines may be divided into two classes: (a) the catecholamines (adrenaline and noradrenaline) which act directly on the blood vessel wall; and (b) the phenylethylamines (e.g. tyramine) which presumably act by liberating a noradrenaline-like substance from depots. The question still remains open, whether the vasoconstrictor effect of 5-hydroxytryptamine (5-HT) can be incorporated in one of the two above-mentioned classes or has a character of its own.

Studies were made in dogs anaesthetized with Numal and under ganglionic blockade with chlorisondamine. The blood pressure reaction of intravenously injected 5-HT, tyramine and noradrenaline were recorded before and after adequate treatment with reserpine, cocaine and other substances.

Reserpine and cocaine inhibited the pressor reaction of tyramine and enhanced that of noradrenaline. The vasoconstrictor effect of 5-HT was inhibited by reserpine and high doses of cocaine. Low doses of cocaine, however, potentiated the 5-HT effect. Noradrenaline infusion in reserpine-treated animals restored the action of tyramine, but not that of 5-HT. These and additional findings with other substances as, for example, local anaesthetics, suggest that the 5-HT-vasoconstriction has a certain character of its own and that 5-HT acts at a level intermediate between the site of action of noradrenaline and tyramine.

547 Ability of Vasoconstrictor Drugs to Cause Adrenal Medullary Discharge after "Sensitization" by Ganglion Stimulating Agents. J. W. McCubbin, Y. Kaneko and I. H. Page (U.S.A.).

Administration of 1:1-dimethyl-4-phenylpiperazinium iodide, nicotine or tetramethylammonium iodide subcutaneously, intravenously or into a lumbo-adrenal artery for 20 or more min, and in dosages that elicited little or no change in arterial pressure, caused the adrenal to respond to intravenous or local arterial injection of small amounts of various vasoconstrictor drugs by discharging large amounts of pressor material that raised dogs' arterial pressures by from 50–180 mm Hg. This sensitizing effect of the ganglion stimulating agents persisted for one or more hr after discontinuing their administration. After sensitization, partial constriction of a lumbo-adrenal artery, or injection of potassium cyanide into the artery, produced responses comparable to those due to vasoconstrictor drugs; carotid occlusion or electric stimulation of the cut peripheral end of a splanchnic nerve did not cause the same large adrenal discharge. Arterial constriction or drug injection did not have a significant effect on arterial pressure prior to treatment with a ganglion stimulating agent. Response to vasoconstrictor drugs injected into a lumbo-adrenal artery was prevented by simultaneous administration of a vasodilator drug. The sensitizing effect of the ganglion stimulating agents was not prevented by atropine, spinal cord section or splanchnic nerve section. Sensitization was eliminated for prolonged periods by injection of very small dosage of TEAC or phentolamine into the lumbo-adrenal artery.

It is concluded tentatively that ganglion stimulating agents cause sensitization of a neurogenically mediated, local adrenal mechanism that responds to hypoxia by causing discharge of large amounts of pressor material, presumably catecholamines.

548 Studies on the Cardiovascular Actions of Antisympathomimetic Drugs. B. G. Benfey and D. R. Varma (Canada).

It has been reported that the anti-sympathomimetic drugs phenoxybenzamine, dibenamine, piper-

oxane, and phentolamine increase the urinary output of adrenaline and noradrenaline in dogs and cats,[1] and that phenoxybenzamine stimulates the heart and elevates the blood pressure of dogs in ganglionic blockade, which is prevented by pretreatment with reserpine or guanethidine.[2] The present experiments have been carried out both to support the evidence that anti-sympathomimetic drugs release adrenaline and noradrenaline, and to show that these drugs possess a direct hypertensive action. Cats and dogs in barbiturate anaesthesia were used, and the contractile force of the exposed heart was recorded by a Cushny myocardiograph. The drugs were injected either following transection of the cervical spinal cord and vagotomy, or during ganglionic blockade. Initially, all anti-sympathomimetic drugs depressed cardiac contractility, however prolonged cardiac stimulation eventually ensued. Phenoxybenzamine (2–10 mg/kg) and dibenamine (10–20 mg/kg) stimulated the heart of the spinal dog and cat. Piperoxane (2·5–5 mg/kg) and phentolamine (0·25–0·5 mg/kg) stimulated the heart of the cat during ganglionic blockade. Following reserpine (1·5 mg/kg given on two days before the experiment) phenoxybenzamine, dibenamine, piperoxane, phentolamine, and hydergine (0·015–0·03 mg/kg) had strong hypertensive actions in the cat during ganglionic blockade, and there was no cardiac stimulation. The studies are being continued to determine the significance of the results for the anti-sympathomimetic effect.

(Supported by a grant from the Medical Research Council of Canada.)

1. Benfey *et al.* (1959), *Brit. J. Pharmacol.*, **14**, 380.
2. Benfey, *Brit. J. Pharmacol.* In press.

549 Effects of Vasoactive Drugs on Blood Flow and Work Performance in Skeletal Muscle. R. R. Sonnenschein and L. Hirvonen (U.S.A.).

In cats under chloralose–urethane anaesthesia, observations were made of the arterial flow to the gastrocnemius–soleus muscles, systemic and local perfusion pressures, and maximum force produced by isometric contractions of the muscles stimulated at 21 sec through the tibial nerve. Care was taken to eliminate collateral circulation to the muscles being studied and to assure that flow to other muscles and to skin was not included. Reductions in flow by intra-arterial infusion of epinephrine or norepinephrine, clamping of the arterial inflow, or stimulation of the sympathetic chain yielded equivalent reductions in muscle force. Intra-arterial infusion of acetylcholine or of histamine at rates insufficient to produce systemic effects resulted in either of two patterns of response: increase in flow with no change in muscle force, or no change in flow with diminution in muscle force. On the assumption that the maximal muscle force is an index of nutritional flow, these inferences may be drawn: (1) norepinephrine and epinephrine produce parallel decreases in nutritional and non-nutritional flow as is the case with sympathetic stimulation and clamping of the arterial inflow; and (2) acetylcholine and histamine increase non-nutritional flow and/or decrease nutritional flow, providing, of course, that no direct effect is produced on the muscle or on neuro-muscular transmission.

550 Responses of the Human Vascular System to Adrenaline and Noradrenaline in certain Functional Circulatory Disorders. K. Graf and G. Ström (Sweden).

Measurements were made of the effects of intravenous and local intra-arterial infusions of adrenaline (A) and noradrenaline (NA) at different dosage rates on the heart rate, arterial blood pressure and blood flow in the forearm and hand (using venous occlusion plethysmography).

A group of normal subjects (NS) were compared to a group of patients with vasoregulatory asthenia (VA) who showed a hyperkinetic circulation at rest as well as during muscular work along with indirect signs of an increased cardiac sympathetic tone. Studies were also made on a group of patients with postural hypotension (PH) who showed a more or less complete disappearance of the nervous sympathetic tone and of the reflex control of the circulatory system.

In VA, there is a high blood flow and a low vascular resistance in the resting forearm with a relatively high sensitivity to A and low to NA. In PH, the resting forearm blood flow equals the average norm and there is a relatively high sensitivity to A as well to NA.

551 Biological Inactivation by Oxidases and Time Course of Drug Action. H. Blaschko (United Kingdom).

Although the principal task of the enzyme-catalyzed oxidation reactions in mammals is to serve the energy requirements of aerobic cells, a number of oxidases have been evolved which have as their main function the inactivation of substances of high pharmacological activity. The role of these enzymes in the catabolism of the catechol amines, of 5-hydroxytryptamine and of histamine is well known. The inactivation of these substances and their metabolic products, as far as it is brought about by these oxidases, is probably an exclusively intracellular process. The significance of the intracellular localization of amine oxidase and histaminase for the time course of inactivation will be discussed.

More recently, extracellular amine oxidases have been discovered in the blood plasma of many mammals. *In vitro* these enzymes act on many substrates of the intracellular oxidases. Their distribution in different mammalian species and their substrate specificity has led to the suggestion that the principal substrates of the extracellular

oxidases *in vivo* are not the amines attacked by the intracellular catalysts, but other amines (e.g. spermine or spermidine) which are taken up from the lumen of the gastro-intestinal tract. Some of the natural substrates of these plasma oxidases have not yet been identified.

552 Phylogenetic Origin of Detoxication Mechanisms. B. B. BRODIE (U.S.A.).

Comparative studies indicate that the microsomal enzymes in liver with catalyse the oxidation and glucuronide formation of drugs might not be enzymes of intermediary metabolism. They can be considered as part of the biochemical changes that occurred with the emergence of animals on to land. Animals had to give up exposed semi-permeable membranes through which they disposed of lipid-soluble foreign compounds ingested in food. Another way of disposing of non-polar foreign compounds was achieved by the development of a number of non-specific enzymes which convert lipid-soluble foreign compounds to polar substances excretable by the kidney. A drug is therefore a paradigm of a class of compounds to which the organism has been exposed over the ages.

553 Plasma Phospholipid Transfer across the Aortic Intimal Surface in Cockerels made Hyperlipaemic by Stilboestrol Treatment or Cholesterol Feeding. S. CHRISTENSEN (Denmark).

Previously, an experimental procedure for the study of the rates of transfer of phospholipid (and phosphoprotein) across the intimal surface was described.[1] Normal and stilboestrol-treated cockerels were used. For the calculation of the rates of transfer across the intimal surface, use was made of a transfer coefficient which is believed to express the number of μl. of plasma which contain an amount of phospholipid (phosphoprotein) equal to that passing the surface per cm^2 per hr. For both the phospholipid and phosphoprotein the average value for this coefficient was close to 0·10 μl./cm^2/hr in both normal and stilboestrol-treated cockerels. Range: 0·019–0·33 μl./cm^2/hr.

In this abstract the results of a similar study concerned with phospholipid in cholesterol-fed cockerels are reported. The average of the transfer coefficient found in 15 cockerels was 0·113 μl./cm^2/hr. Range: 0·03–0·21 μl./cm^2/hr.

The rate of the transfer of phospholipid in the individual cockerels was calculated as the product of the individual transfer coefficient and plasma phospholipid concentration. When plotting the 15 products thus obtained against aortic cholesterol concentration (range: 2·88–9·26 mg cholesterol/g wet wt.), a linear relationship was found. The correlation coefficient, r, was + 0·94 ($p < 0·001$); if aortic phospholipid concentration (range 6·62–10·7 mg/g) was used the value for r was + 0·74 ($p < 0·01$).

1. CHRISTENSEN, S. (1961), *J. Ather. Res.*, **1**, 140.

554 Dietary Fatty Liver Prevention in Rats by Some Testosterone Derivatives. F. GALLETTI (Italy).

György *et al.* reported that oestrone had a protective action against the development of fatty liver induced in rats by a high fat–low protein diet, while testosterone propionate, given orally, did not show this lipotropic effect.

In the present study two testosterone derivatives were given parenterally, both at 12 μM dosage, dissolved in 0·2 ml of sesame oil, once a day for 15 days to male rats fed with a diet similar to those used by György.

The first compound was testosterone oenanthate (TE), namely a full androgen. The other was the N-heptyl enol ether of testosterone oenanthate (TEn-7), which, administered subcutaneously, revealed no appreciable androgenic and myotrophic activity, while it retained anti-mammary cancer action both experimentally and clinically.

As shown in Table I both testosterone oenanthate and its N-heptyl enol ether significantly prevented the increase of liver fat. Comparable results have been obtained likewise with other testosterone oenanthate enol ethers.

Table I

Treatment	Daily dose (mg)	Liver weight[1] (g)	Total liver lipids (mg)	Total liver lipids (per cent)
Stock diet	—	9·77	360 ± 19	3·68 ± 0·10[2]
High fat diet	—	8·08	824 ± 185	10·38 ± 1·51
High fat diet + TE	4·889	8·10	438 ± 32	5·48 ± 0·36
High fat diet + TE N-7	5·976	8·30	480 ± 20	5·72 ± 0·16

1. Adult Sprague Dawley male rats, six each group.
2. Standard error of the mean.

555 Action of Triton WR-1339 on the Intestinal Absorption of Fat. M. KRAMER and A. GIOIA (Italy).

The hyperlipaemic action of Triton WR-1339 (a polymer of *p-iso*octylpolyoxyethylenephenol) is due, according to several authors, to an increase of cholesterol, fatty acid, and phospholipid synthesis in the liver. Other workers assign more importance to the change of the physical-chemical properties of the serum proteins or to a supposed increase of lipid mobilization. We injected in rats 200 mg/kg Triton intravenously with and without simultaneous oral administration of 2 ml/100 g olive oil. The animals were sacrificed 18 hr later and

determinations were made of the serum level of free and total cholesterol, phospholipids and total lipids. The most important results obtained from groups of 6 rats each are reported in the following table:

treatment	dose	esterified cholesterol mg %	total lipids mg %
controls	—	53·4 ± 11	110 ± 30
olive oil	2 ml/100 g	78·8 ± 40	680 ± 490
Triton WR-1339	200 mg/kg	96·3 ± 21	1120 ± 300
olive oil	2 ml/100 g	225·7 ± 60	6260 ± 1800
Triton WR-1339	200 mg/kg		

The results indicate that Triton favours enormously the intestinal absorption of fat and that the increase of lipid and cholesterol synthesis seems to play a less important role for the hyperlipaemic action of this substance.

556 In Vivo Studies on the Nature of the Lipemia Clearing Factor. G. E. Cronheim (U.S.A.).

Lipaemia clearing factor is found in mammalian circulation after injection of heparin and synthetic heparinoids. The present investigation was concerned with the biological properties and the role of clearing factor in lipid metabolism.

The experimental evidence is based on a comparison of heparin with SPG, a synthetic sulphated glucose polymer. Clearing activity, plasma triglycerides and cholesterol, metachromasia, blood clotting time and other parameters were measured in dogs and other animals. Single and repeated, parenteral and oral doses were given without and with alimentary lipaemia. Radioactive (^{35}S) heparin and SPG were used for metabolic studies.

Both heparin and SPG induced lipaemia clearing activity in the plasma of all species tested, increased free fatty acid, and also prolonged whole blood clotting time. Both drugs were active when given by injection, and also when administered orally together with certain adjuvants, such as edathamil sodium. Heparin had a relatively greater anticoagulant and SPG a relatively greater antilipaemic activity. Furthermore, the antilipaemic time–effect curves for the two drugs differed in rapidity of onset and in duration. In contrast, no differences existed in the anticoagulant time–effect curves of heparin and SPG. After i.v. injection of SPG labelled with ^{35}S, radioactivity, metachromasia and anticoagulant effect disappeared from the blood at the same rate, while clearing activity could be demonstrated for much longer. This suggests that more than one mechanism (or enzyme) is involved in clearing activity.

In lipaemic patients, oral SPG with edathamil sodium lowered serum triglycerides, cholesterol and lipoproteins, and this reduction persisted for some time after discontinuing the drug. Thus, providing additional clearing activity may have desirable therapeutic effects.

557 Lipid Clearing Effect of Acid Mucopolysaccharide Extracts on Arterial Tissue. L. M. Morrison, J. J. Quilligan, O. A. Schjeide, L. Freeman and R. Holeman (U.S.A.).

Tissue culture systems have been utilized for the past two years to bio-assay the lipid-clearing action of acid mucopolysaccharides upon animal and human aortic connective tissues and cells.

Various cell lines and systems have been studied to evaluate the therapeutic effect of AMP (acid mucopolysaccharides) upon aortic and arterial cells and tissues.

Photomicrographs in colour will be shown demonstrating the technique, time factors and biochemical significance of the pharmacological activities involved in the cellular systems of this phenomenon. This research is reported and presented here for the first time. These tissue culture studies demonstrate that AMPs have the ability to clear lipids such as cholesterol, neutral fats and the total serum lipids found in human blood serum from animal and human arterial wall cells. This technique is presented as a method of bio-assay for use in research on atherosclerosis and arteriosclerosis.

Particular reference is made to the AMPs chondroitin sulphate A, B and C, hyaluronic acid, heparins and heparinoid-like substances in these studies.

558 The Effect of Ethanolamine on Changes in Lung Lipids Induced by Anaphylaxis. W. G. Smith (United Kingdom).

It has recently been reported from this laboratory that ethanolamine inhibits the release of SRS-A (slow reacting substance of anaphylaxis) in sensitized guinea pig lungs injected with specific antigen.[1] The known effects of ethanolamine on phospholipid synthesis and phospholipid turnover thus prompted an investigation of the effects of anaphylaxis on the lipid content of guinea-pig lungs and the influence thereon of anti-anaphylactic doses of ethanolamine. Anaphylaxis caused extensive alterations in the lipid content of sensitized lungs, especially the triglyceride, cephalin, lecithin, and sphyngomyelin fractions. Lungs from animals which had been pretreated with ethanolamine and which had liberated no SRS-A during anaphylactic shock showed much smaller alterations in their lipid content. Fractionation of the extracted lung lipids was accomplished by chromatography on columns

of silicic acid by the technique described by Turner *et al.*[2]

1. Smith, W. G. (1961), *J. Pharm. Pharmacol.*, **13**, 1.
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559 Central Action of Certain Drugs upon Gastric-wall Dystrophy. I. S. Zavodskaya (U.S.S.R.).

The aim of the present study was to show the mechanism of action of central neurotropic drugs in preventing reflex dystrophy and gastric-ulcer formation induced by excessive irritation of the duodenum. The experiments were carried out on rats and guinea pigs.

It is demonstrated that this action is not ascribable to inhibited gastric-juice secretion, as the preventive effect upon reflex impairment of trophic processes in the gastric wall stands in no relation to the intensity of the inhibitive action of the drug.

Thus, barbiturates (such as luminal) and certain other anticonvulsants (Dilantin, trimethadione) may, in appropriate dosage, produce the same action upon gastric uice secretion, but luminal is effective, and Dilantin and trimethadione ineffective in preventing reflex dystrophy. The same difference exists between various classes of central cholinolytics.

In doses exerting the same inhibitive effect upon gastric-juice secretion, cholinolytics neutralizing arecoline action (e.g. benactyzine) are more effective in preventing reflex dystrophy than those neutralizing nicotine action (e.g. trasentin).

An analysis of the EEG obtained enables the author to draw the conclusion that, in the mechanism of central neutropic-drug action upon reflex gastric-wall dystrophy, the most important part is pplayed by the blocking effect upon the upper part of the reticular formation.

560 Mechanism of Ulcerogenic Action of 5-Hydroxytryptamine in Rat's Stomach. B. Nikodijević and T. Trajkov (Yugoslavia).

It is a well known fact that 5-HT administered subcutaneously produces gastric erosions and ulcerations in rats. It is also known that LSD-25, BOL-148 or some of the antihistaminic drugs (which have marked antiserotonin properties) prevent the gastric ulcerations normally provoked by serotonin. It was recently shown that iproniazid also prevent serotonin gastric ulcers in mice.

Our experiments were performed on 53 albino rats of both sexes of 110–150 g body wt., divided into four groups. The first group of 6 animals served only for control determinations of the concentration of histamine ($22{\cdot}5 \pm 2{\cdot}4\ \gamma$/g) and serotonin ($1{\cdot}45 \pm 0{\cdot}07\ \gamma$/g) in the stomach. The second group of 16 animals was treated with 5-HT 20 mg/kg subcutaneously twice on one day only; the animals were sacrificed 6 hr after the second injection. There was an ulcer index of 11; the concentration of histamine in these rats' stomachs was $15{\cdot}3 \pm 1{\cdot}8\ \gamma$/g, and of serotonin $3{\cdot}06 \pm 0{\cdot}25\ \gamma$/g. The third group of 16 animals was given Antistin (30 mg/kg intraperitoneally four times) on one day, and 5-HT in the same doses and in the same way as the previous group. There was a 4·5 ulcer index; histamine $24{\cdot}7 \pm 6\ \gamma$/g of stomach, and serotonin $3{\cdot}38 \pm 0{\cdot}123\ \gamma$/g. The fourth group of 15 animals was pretreated with iproniazid 100 mg/kg i.p.; 10 hr later ulcerogenic doses of 5-HT were administered. There was an ulcer index of 9·2; histamine—$18{\cdot}2 \pm 3{\cdot}2\ \gamma$/g of stomach and serotonin—$9{\cdot}38 \pm 0{\cdot}46\ \gamma$/g.

With regard to the above results, we think that the following may be stated: (1) Antistin (an antihistaminic drug which has almost no antiserotonin properties), considerably reduces the 5-HT provoked gastric ulcers in rats; (2) Iproniazid did not show visible protective effect on gastric ulcer formation; and (3) ulcerogenic action of serotonin on rat's stomach is the result of the histamine liberating properties of this substance rather than of a direct 5-HT effect on the rat's gastric mucosa.

561 Structure–Activity and Enzyme–Substrate Relationships of Histamine, Histamine Metabolites and Analogues in Stimulation of Gastric Secretion. T. M. Lin, F. G. Henderson, K. K. Chen and D. N. Benslay (U.S.A.).

The structure–activity relationship of 6 histamine metabolites and 2 imidazole derivatives (10-pyrazole, 13 β-triazole), 2 tetrazole analogues of histamine and 6 miscellaneous β-aminoethyl compounds on gastric HCl secretion was compared in dogs. The *in vitro* susceptibility of these agents to histaminase and its relation to *in vivo* potentiation of their effects by a histaminase inhibitor were also studied. Linear dose–response relationship was obtained by oral administration of all effective agents resistant to the action of histaminase. Contrary to histamine, the action of these agents on gastric secretion was not augmented by the histaminase inhibitor. The side effects of these products resembled those caused by histamine. Antihistaminics blocked the side effects of histamine and its derivatives but had either no effect or augmented the gastric response of the dog or cat to the same substances. It is postulated that histamine and its metabolites or analogues act on the same receptor sites; the receptor sites on the parietal cells are distinct from the receptor sites for histamine in other organs or tissues. The significance of these findings will be discussed.

562 Reserpine and Gastric Acid Secretion. S. Emås (Sweden).

The mechanism of the stimulating effect of

reserpine on gastric secretion is unknown. Several explanations are possible. For example, reserpine may: (a) act directly on the acid secreting cells; (b) liberate histamine; or (c) eliminate the gastric secretory inhibitor serotonin. Reserpine causes the release of catecholamines from central and peripheral nervous structures. This results in (d) a decreased activity of the sympathetic nervous system which may influence gastric secretion.

In non-anaesthetized gastric fistula cats[1] it has been demonstrated that daily reserpine injections result in enhanced spontaneous secretion and increased secretory responses to histamine and gastrin but lowered responses to insulin hypoglycaemia.[2]

To test the role of the peripheral sympathetic nervous system, gastric fistula cats were submitted to extirpation of the coeliac ganglia and to sympathectomy. Following each operation the secretory responses to histamine and gastrin were compared before and during prolonged reserpine treatment (0·1 mg/kg body wt. daily intramuscularly). Neither the enhanced spontaneous secretion nor the increased secretory responses during reserpinization seemed to be influenced by the denervations.

Reserpine infused intravenously for 15 min in non-anaesthetized cats elicits an acid secretory response usually within one hour which lasts for at least four hours. This secretory response is not influenced by the operations mentioned. The serotonin content of gastric mucosa remained unchanged during reserpinization.[3]

Further experiments are in progress. The site of action of reserpine on gastric secretion will be discussed.

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2. Emás, S. (1960), *Acta Physiol. Scand.*, **50,** Suppl. 175, 38.
3. Emás, S. and Tham, R. To be published.

563 Influences of Posture on Gastric Function: Absorption of Glucose and Production of Acid. R. A. Lewis and D. Said (Iraq).

Most studies of gastric function are concerned with motility or secretion. However, since many drugs are absorbed from the stomach, it is also important to evaluate absorption. We have studied the absorption of glucose and the secretion of acid in 10 essentially normal adult males, and have compared results obtained in the sitting position with results obtained in the same individual while standing.

We have employed the conventional oral glucose tolerance test, measuring the blood level at hourly intervals after the administration of 1 g/kg glucose in 10 per cent solution. At the same time we have measured the hourly urinary excretion of azure A liberated from orally administered resin (tubeless gastric analysis).

There was a uniform and statistically significant difference in the peak of the glucose level, which was 52 mg per cent above fasting when the individual was seated.

These studies require further elucidation and clarification. Nevertheless, one can conclude that for both the diagnostic tests employed, posture must be taken into account. Furthermore, posture may affect the rapidity and completeness of absorption of orally administered drugs. Finally, unless and until it is shown that digestion is normal in the standing position, it would be safer to sit while eating.

564 Mode of Action of Chlorpromazine. P. S. Guth and M. A. Spirtes (U.S.A.).

The number of reports concerning the involvement of chlorpromazine (CPZ) with membrane phenomena grows steadily. Some of the membranes studied involved striated muscle, erythrocytes, blood brain barrier, mitochondria, intestine and synaptic vesicles. In most cases permeability to specific substances seems to be decreased by the presence of CPZ. Mitochondria, however, appear to exhibit opposite effects at high and low concentrations.

If membranes are involved in CPZ action then two alternate hypotheses may be presented to account for its specific, circumscribed central nervous system effects: (A) that certain areas of the brain concentrate CPZ and subject membranes within these areas to supra-effective concentrations while other (unresponsive) areas contain sub-effective concentrations; (B) that certain, perhaps unique, membranes are unusually sensitive to the effect of CPZ.

Hypothesis A is supported by data showing that certain brain areas concentrate CPZ, e.g. after CPZ injection, the hypothalamus has been shown to contain 10^{-4} M CPZ or more. Such a tissue concentration statement does not necessarily indicate the concentrations in and around intracellular structures. Thus, the mitochondrial function of brain has been shown to contain 65 per cent of the total CPZ in a homogenate. Likewise, isolated mitochondria remove CPZ from solution and concentrate it to the extent of 200 μg/mg mitochondrial N or more.

Hypothesis B finds support from experiments with isolated synaptic vesicles. Synaptic vesicles appear to be found only in the nervous system. The release of acetylcholine by these organelles is inhibited by as little as 5×10^{-6} M CPZ.

565 Effect of Largactil on Central Nystagmus. F. Bergmann, J. Lachmann, M. Chaimovitz and J. Gutman (Israel).

The nystagmus response, evoked by electrical stimulation in the nystagmogenic centre of the mesodiencephalon of the rabbit,[1] is influenced in a

characteristic way by intravenous administration of small doses of largactil (0·2–0·3 mg/kg): (a) After-nystagmus is considerably enhanced; (b) The decay of the response under high frequency stimulation[2] is delayed or completely abolished; and (c) The maintenance of a uniform, persistent reaction during intermittent stimulation is greatly improved.

The effects can be tentatively explained by assuming either a selective suppression of the inhibitory components of the nystagmus mechanism or a specific facilitation in reverberating circuits.

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566 The Neuroplegic Effect of Frenolon, a New Phenothiazine Compound. J. Borsy and L. Toldy (Hungary).

We made investigations into the neuro-sedative and toxic actions of the above compound and compared the results with those exhibited by chlorpromazine, perphenazine and thiopropazate, respectively.

With intraperitoneal or peroral administration in mice, Frenolon strongly inhibits the hyperactivity due to the orientational reflex. Its 50 per cent inhibitory dose (ED_{50}) amounts to 0·23 mg/kg i.p. thus being 6·07 times as active as chlorpromazine in this respect. By the oral route Frenolon proved to be 3·1 times as active as chlorpromazine, it exceeded the activity of perphenazine and nearly equalled thiopropazate.

When using Schlichtegroll's method, Frenolon exerted strong cataleptic action, its 50 per cent cataleptic dose (ED_{50}) being as high as 5 mg/kg per os in the mouse. Concerning its catalepsy producing efficacy, this is somewhat higher than the other phenothiazine derivatives examined by us.

By means of Burn's method, Frenolon, in doses of 2·5–5·0 mg/kg by mouth, diminished the toxicity of benzopropamine. The hypermotility state evoked by this excitant drug in mice was depressed by an i.p. dose of 0·25 mg/kg of Frenolon (60 per cent inhibitory effect), whereas chlorpromazine was able to inhibit only to 46 per cent in a dose of 1·0 mg/kg.

Frenolon potentiates Hexobarbital anaesthesia in rats to the same extent as perphenazine or thiopropazate. Its hypothermic effect is weaker than that of both the latter drugs.

Frenolon seems to possess low grade, acute and chronic toxicity by intravenous and peroral administration, respectively.

567 On the Relation between the Ataractic and Some Other Types of Central Action of Some Phenothiazine Derivatives. B. I. Liubimov and K. S. Raevsky (U.S.S.R.).

This work has been undertaken for the purpose of comparing various aspects of central action exerted by neuroplegic drugs of the phenothiazine group by means of an assessment of the intensity of such effects exerted by each agent. The intention was also to find out whether any parallelism existed between the ataractic, the anticonvulsant, the central muscle-relaxant and the hypothermic effects of these agents. Aminazine, propazine, acetazine, mepazine, meterazine and etaperazine were the objects of this study. Luminal, possessing a marked depressing effect on central nervous system, was chosen for the sake of comparison. White mice and rats served as experimental animals. The experiments have shown that etaperazine and meterazine possess the most marked ataractic effect, propazine and mepazine the least, with acetazine and aminazine occupying an intermediate position. Luminal, according to our data produces no ataractic effect. The anticonvulsant effect was equally marked in propazine, aminazine, acetazine and mepazine. Meterazine and etaperazine possess low activity in this respect. Central relaxant action was most pronounced with acetazine and aminazine, next with etaperazine and meterazine while propazine and mepazine were least active in this respect. Hypothermic action of the above agents in doses corresponding to ED_{50} of the ataractic effect manifested itself in the following manner: the greatest temperature drop (7°) was seen after administration of acetazine and propazine; aminazine and mepazine depressed the body temperature of animals by 3–4°; and, finally, meterazine and etaperazine in the above doses were practically unaccompanied by any hypothermic effect. In doses corresponding to ED_{50} of the muscle relaxant effect, propazine, aminazine, mepazine and acetazine produced considerable hypotoermia: body temperature of animals dropped by 7–11°. Meterasine and etaperazine produced a drop of 2·5–4·5°.

Summarizing our data, one may conclude that in some of the substances (etaperazine and meterazine) the ataractic effect is pronounced, the anticonvulsive action is practically absent, and the hypothermic and the central relaxant effects are moderate. In others (mepazine and propazine) the anticonvulsive action is intense, while the ataractic and the muscle relaxant effects are much weaker than in the related substances.

The results obtained show that in this series of phenithiazine derivatives no parallelism exists between the various type of central action studied.

568 On the Influence of the Neuroplegic Drugs on Chemoreception. U. I. Vikhliaev (U.S.S.R.).

The author studied the influence of a number of phenothiazine derivatives, aminazine, mepazine, propazine and etaperazine (Trilafon), administered intravenously and intra-arterially, on the sensitivity of the blood vessels in the hind-leg to acetylcholine and NaCl. Experiments were carried out on anaesthetized cats (uretrane, urethane with chloral), using the method of vascular isolation of the lower

limb (with preservation of neutral pathways) perfused by blood from a donor animal. Blood pressure of the donor and the recipient was recorded, and in certain experiments the perfusion pressure in the blood vessels of the isolated extremity also was recorded.

Aminazine, propazine, mepazine and etaperazine given to the donor intravenously (in doses up to 10 mg/kg) exerted no appreciable effect in chemoreception. Suppression of chemoreceptors was obtained by direct administration of these agents into the arterial bed of the isolated extremity. Thus, neuroplegic drugs by the usual routes do not influence materially the excitability of the chemoreceptors. Hence, intravascular anaesthesia plays apparently no significant role in the mechanism of depressor action of these agents on the vascular reflexes.

569a Photochemische Umwandlung von Phenothiazinen. G. URBAN (Deutschland).

In Lösung befindliche Substanzen von Phenothiazinstruktur, welche therapeutisch benutzt werden, wurden im Experiment durch Bestrahlung mit sichtbarem oder ultraviolettem Licht in Kürze zu stark gefärbten Verbindungen oxydiert. Durch Zusatz von reduzierenden, körpereigenen Stoffen konnte inhre Photooxydation verhindert werden. Die erhaltenen Befunde werden zur Photoallergie, die nach therapeutischem Gebrauch von Phenothiazinen beobachtet wird, in Beziehung gesetzt.

569b Photochemical Conversion of Phenothiazines. G. URBAN (Germany).

Solutions of substances with a phenothiazine structure used therapeutically were soon oxidized experimentally by irradiation with visible or ultraviolet light- to heavily-coloured compounds. Addition of reducing substances of the body prevented their photo-oxidation. The findings obtained are related to the photo-allergy observed after therapeutic use of phenothiazines.

570 Failure of Ethanol and Barbiturates to Alter the Content of Brain 5HT and NE. D. H. EFRON and G. L. GESSA (U.S.A.).

There are numerous contradictory reports in the literature concerning effects of ethanol and barbiturate administration on brain 5HT and NE content. According to one report, ethanol increases brain 5HT content in rats, while another report describes a dramatic decline of brain 5HT and NE content in rabbits given ethanol either chronically or acutely. Other reports state that pentobarbital and phenobarbital cause an increase of brain 5HT content assayed using the heart of *Venus mercenaria*. Because of the important implications of these findings, it was thought desirable to investigate further the effects of potent depressants such as CNS ethanol and barbiturates on brain amine content using specific spectrophotofluorimetric techniques. Brain 5HT and NE content of rabbits or rats given ethanol or barbiturates was determined. Although the experimental conditions (doses, route of injection, animal species, etc.) were kept similar to those of the above-mentioned experiments, in our hands neither barbiturates nor ethanol, altered the brain content of NE and 5HT. An interference by barbiturates with the effects of 5HT was found in the heart of *Venus mercenaria*, which might account for the apparent increase of brain 5HT when this biological preparation is used to assay 5HT. However, in the case of ethanol a cause for the discrepancy between our negative results and the positive reports mentioned above could not be identified.

571 Metabolic Effect of Ethanol in Small Doses. E. S. PERMAN (Sweden).

Rabbits pretreated with glucose and anaesthetized with urethane were used. The oxygen consumption was continuously determined by the Benedict technique and the animal was connected to a closed-circuit system via a tracheal cannula. Intravenous infusion of 0·2 g/kg ethanol (diluted in saline) increased the oxygen consumption 10–18 per cent. The increase lasted about 1 hr. The peak blood ethanol concentration was only 0·02 per cent in one experiment of this type. Increased oxygen consumption was seen in the dose-range 0·1–0·3 g/kg. Higher doses usually caused no further increases in rectal temperature were noted in some experiments but the respiration and blood pressure were mainly unaffected. The blood glucose concentration increased after ethanol. Similar results were obtained during artificial ventilation of the animal after curarization indicating that the effect is not due to increased muscular activity. Ganglionic blocking with hexamethonium or bilateral splanchnic section did not abolish the oxygen consumption increase indicating that it is not primarily caused by increased adrenal medullary secretion. It is well known that ethanol is rapidly metabolized by the organism. The present results suggest that ethanol in a low dose-range increases the metabolic rate.

572 Electrically Induced Changes in Energy-Rich Phosphates of Brain Slices as Modified by Ethanol. H. WALLGREN (Finland).

Some known metabolic changes induced by ethanol in electrically-stimulated brain slices are the decreased rates of respiration, of glucose utilization and of lactic acid formation. Studies with metabolic inhibitors and pharmacological agents indicate that ethanol may act directly on the excitable membranes causing a decrease in the utilization of energy-rich phosphates and thus retardation of mitochondrial oxidations. This implies that the rapid initial break-down of ATP and creatine phos-

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phate at the application of electrical pulses would be slower in the presence of ethanol. Experiments based on the above hypothesis will be reported.

573 Effect of Ethanol on the Di- and Triphosphopyridine Nucleotide Content in Rat Liver. N. C. R. RÄIHÄ (Finland).

It has been shown previously[1, 2] that a lowering of the DPN/DPNH ratio in the liver is brought about during ethanol oxidation. The lowering of the ratio is primarily due to an accumulation of DPNH. Fasted animals show a decreased rate of ethanol oxidation which can be increased to normal by agents which re-oxidize DPNH. It is known that certain steroid hormones (pyridine nucleotide transhydrogenases) demonstrated in placental and liver tissue, function as coenzymes in transfer of hydrogen between DPN and TPN. It can thus be postulated that the oxidation of ethanol which shifts the DPN/DPNH ratio towards the reduced form might affect pathways using the TPN system and through a transhydrogenase produce changes in the TPN/TPNH ratio.

Studies on the effect of ethanol on the DPN/DPNH and TPN/TPNH content of rat liver will be presented and discussed.

1. FORSANDER, RÄIHÄ and SUOMALAINEN (1958), *Zeitschr. Physiol. Chemie.*, **312,** 243.
2. SMITH and NEWMAN (1959), *J. Biol. Chem.*, **234,** 1544.

574 The Induction of Alcohol Oxidation and Catalase Sensitivity to 3-Amino 1:2:4-Triazole (AT) in the Intact Erythrocyte. R. E. PARKS, JR., T. TEPHLY and G. J. MANNERING (U.S.A.).

In view of the high catalase concentration of mammalian erythrocytes it is somewhat surprising that these cells, under normal conditions, appear incapable of oxidizing alcohols. Furthermore, in animals injected with 3-amino 1:2:4-triazole (AT), erythrocytic catalase is unaffected while hepatic and renal catalases become drastically inactivated. Recent work in this laboratory suggested that these anomalies might be explained by the failure of erythrocytes, under normal conditions, to synthesize significant amounts of H_2O_2. A means of testing this hypothesis was offered by the finding of Brin and Yonemoto that methylene blue added to washed erythrocytes induces in these cells a marked stimulation of oxygen and glucose uptake through an activation of the hexose monophosphate cycle. Presumably, methylene blue mediates this effect by transporting electrons from flavin enzymes (known to exist in erythrocytes) to O_2. H_2O_2 is a product of flavin enzymatic activity. When methylene blue is added to washed suspensions of rat or human erythrocytes in glucose and phosphate buffer, these cells become capable of oxidizing ethanol and methanol at significant rates. Furthermore, when AT, methylene blue and glucose are added to washed erythrocytes, the catalase of these intact cells becomes inactivated. The kinetics of inactivations resemble those observed when AT is added to a crystalline catalase—purified peroxide generator system. In addition to methylene blue, a number of other susbstances elicit these effects. Potentiations are observed when methylene blue and ascorbic acid are employed simultaneously.

575 Oral Hypoglycaemic Drugs and Ethanol Metabolism. W. C. CLARK, R. J. HAINES and P. A. OWENS (U.S.A.).

The popularity of oral hypoglycaemic drugs, tolbutamide (Orinase) and chlorpropamide (Diabinese) in treating diabetes mellitus renders data concerning their effect on ethanol metabolism desirable. Such information may help to clarify the role of insulin in the metabolism of ethanol. Clark,[1] Newman[2] and others find ethanol metabolism accelerated by insulin. Contrary findings are reported by Hirschfelder,[3] Harger,[4] Kinard[5] and others.

Data obtained in this investigation show that normal dogs had a disappearance rate of ethanol from their blood of 14·6 ± 0·7 mg (standard error) per 100 ml per hr. After tolbutamide 30 mg/kg, a rate of 21·6 ± 0·6 mg, and after chlorpropamide 100 mg/kg, a rate of 19·1 ± 0·7 mg, and after 5 mg/kg of phenformin, a biguanide hypoglycaemic drug, a rate of 16 mg (2 observations).

After diabetes (alloxan and pancreatectomy) the dogs had a higher control rate (mean 18 mg) and after tolbutamide or chlorpropamide they had rates similar to those seen in the dogs following tolbutamide or chlorpropamide treatment before induction of diabetes.

1. CLARK, B. B. *et al.* (1941), *Quart. J. Stud. Alc.*, **1,** 683.
2. NEWMAN *et al.* (1959), *Quart. J. Stud. Alc.*, **20,** 520.
3. HIRSCHFELDER and MAXWELL (1924), *Amer. J. Physiol.*, **70,** 520.
4. HARGER and HULPIEU (1935), *F. Pharmacol.* **54,** 145.
5. KINARD and COX (1958), *Quart. J. Stud. Alc.*, **19,** 375.

576 Hypoxaemia Test for Evaluation of Agents on Experimental Coronary Insufficiency induced by Atherosclerosis in Rabbits. I. I. A. TABACHNICK, L. SZYBALSKI and M. M. WINBURY (U.S.A.).

The hypoxaemia test for the electrocardiographic diagnosis of coronary insufficiency has been employed by Winbury *et al.*[1] for the evaluation of

the state of the coronary circulation of rabbits fed a 2% cholesterol diet. When these animals breathe a 10% O_2 : 90% N_2 gas mixture (Gas I), the resultant hypoxaemia was accompanied by an S-T segment depression. More recently the procedure has been improved by changing to a gas mixture of 6·5% O_2 : 4·5% CO_2 : 89% N_2 (Gas II).

Gas II induced marked S-T segment depressions within 7 weeks on the cholesterol diet. At this time, Gas I did not significantly alter the electrocardiographic contour of cholesterol-fed animals. At the end of 14 and 18 weeks of this diet both gases caused S-T segment depression, increasing progressively at each time period studied. However, Gas II elicited a greater S-T segment depression than Gas I.

The effects of several drugs have been evaluated in cholesterol-fed rabbits breathing Gas II. Triparanol prevented the S-T segment depression during the period of drug administration, whereas a MAO inhibitor, JB 516, had no demonstrable effect.

Concurrent feeding of propylthiouracil with cholesterol reduced the severity of the S-T segment depression.

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1. Winbury *et al.* (1961), *Amer. J. Physiol.*, **200,** 642.

577 Glycine and Glucuronic Acid in Salicylate Intoxication. A. Ghazal and H. N. Wright (U.S.A.).

Dogs anaesthetized with sodium pentobarbital were given a dose of 0·25 g sodium salicylate per kg intravenously, a dose which is fatal within 2–4 hr. Determinations of the blood pressure and pH, plasma salicylate and total carbon dioxide, the respiratory rate and volume, as well as the urine salicylate, were made. Glycine or glucuronic acid was given to the dogs as the salicylate administration was finished. The effectiveness of the use of any of these two substances was compared with other treatment. The results showed that glycine in a bicarbonate–glucose isotonic mixture given intravenously saved three dogs from fatal salicylate effect and the plasma salicylate was decreased by 92 per cent and urinary salicylate excretion was 90 per cent in 12 hr. Glucuronic acid in a bicarbonate–glucose isotonic mixture increases the excretion of salicylglucuronic acid and the plasma salicylate was decreased by about 75 per cent. Two of three dogs were saved and the third lived 11½ hr. This treatment was compared with the use of sodium r-lactate, aminophyline, thiomerin sodium, 20 per cent glucose, sodium bicarbonate and sodium carbonate, and was found to be superior to all of them.

578 The Uptake of Labelled Lipoprotein Fractions by Isolated Human and Animal Arterial Endothelial Cells. A. Lazzarini-Robertson, jr. (U.S.A.).

The transport and metabolism of aqueous microemultions of protein-bound cholesterol-4-^{14}C and ^{3}H by human and animal intimal cells from large arteries of normal and hypercholesterolemic donors were followed in short-term organ cultures. Two different cell types can be distinguished by their lipid uptake and relation of subcellular structures to cytoplasmatic droplets as seen with the electron microscope. A shift of cholesterol-phospholipid complexes from the plasma membrane to the cytoplasm occurs before extracellular lipoproteins are incorporated.

Sodium heparin at concentrations of 5–10μg/ml produce a marked inhibition of this transport. This effect seems to be independent of the known action of heparin on hyperlipaemic plasma and related to changes on the electrical characteristics of the cell membrane.

These findings are in agreement with the studies of Capraro *et al.* on the permeabilizing effect of heparin for Ca ions and von Jancso on phagocytosis of colloidal gold particles. These results strongly suggest that the action of heparin at cellular level is similar to that of other surface-active agents and provide further evidence of the importance of surface charges in cellular transport mechanisms.

(Supported by grants from the American Heart Association, New York Heart Association and U.S. Public Health (H-2471). This work was done during the tenure of an Establish Investigatorship of the American Heart Association.)

579 Differential Effects of Meprobamate on Conditioned Renal and Motor Defence Responses. S. A. Corson, E. O'Leary Corson, R. A. Dykman, J. E. Peters, W. G. Reese and L. D. Seager (U.S.A.).

Conditioned motor defence responses and conditioned antidiuretic responses (CAR) were developed in water-loaded mongrel dogs with implanted cystostomy tubes by pairing tones with electric shocks to a foreleg. The urine secreted during CAR had a high osmolarity (accounted for chiefly by increased urea concentration), suggesting the participation of pituitary antidiuretic hormone. In certain types of dogs these CAR develop rapidly, become intensified with time, and are extremely difficult to extinguish. The CAR are accompanied by marked retention of Na, K and Cl. Similar conditioned electrolyte retention responses (CERR) were developed in hydropenic dogs. Administration of tritiated water demonstrated that the conditioned antidiuresis was not a result of inhibition of the absorption of water from the gastrointestinal tract. Oral administration of meprobamate (20–60 mg/kg/day) abolished or markedly inhibited the CAR and CERR but had no inhibitory influence on the conditioned or unconditioned motor defence responses. Meprobamate also ameliorated the unconditioned antidiuretic responses to electric shock. Control experiments demonstrated that meprobamate *per se* exhibits no diuretic properties in dogs not exposed to stress. In one dog an intense

schedule of experimentation designed to develop conditioned diuretic responses led to the development of a paradoxical marked and sustained antidiuresis in response to the administration of the conditioned stimulus reinforced with a water load. Under these conditions it was impossible to demonstrate any evidence of conditioned diuretic responses. Administration of meprobamate not only eliminated this paradoxical antidiuresis, but permitted the development of conditioned diuretic responses.

579a Reduction of Toxic Effects of Radiopaque Contrast Media with Curare and Procaine. F. M. Bunce (U.S.A.).

A variety of radiopaque media were examined while studying the collateral blood supply to the brain in the dog. Of these, sodium acetrizoate produced the best results radiographically, but had the disadvantage of causing toxic effects which varied in severity according to the site of injection. Administration of 10 ml 70 per cent sodium acetrizoate into the common carotid artery of the anaesthetized dog caused mild clonic convulsions which persisted for 1–3 min. Introduction of the same quantity of the medium into the internal carotid artery provoked a very severe response characterized by violent tonic-clonic convulsions and marked extensor rigidity which would terminate fatally in 5–10 min unless artificial respiration was begun. When the medium was administered into the vertebral artery, violent tonic-clonic convulsions occurred during the injection followed by death from respiratory arrest within 1–3 min despite artificial respiration. These results indicate that the material may have passed through the blood-brain barrier to affect directly the medullary respiratory centre.

In an attempt to reduce convulsive activity, and obtain better radiographs, 3 ml 1 per cent procaine were injected into the vertebral artery followed by 10 ml 70 per cent sodium acetrizoate. Mild clonic-type convulsions occurred resembling those which followed injection of the medium into the common carotid artery. Similar results were obtained substituting 3 mg tubocurarine chloride for procaine. However, when a combination of procaine and curare were administered previous to injection of the contrast medium, toxic manifestations included only mild muscular twitching and a decreased respiratory rate. This protective effect continued for 2 hr, even though the medium was re-injected at 10–20 min intervals.

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580 Bradykinin and Vaso-Dilating Polypeptides. Introductory Remarks. U. S. von Euler (Sweden).

581 Bradykinin and Vasodilating Polypeptides. M. Rocha e Silva (Brazil).

The name "Kinin" suggested by the English authors might be useful by applied to the class of tissue hormones of a polypeptide nature acting upon smooth muscles and having vaso-active properties (either increasing or decreasing blood pressure). This definition would exclude the neurohypophysis hormones, but would include the angiotensins and possibly also substance-P. A characteristic of the "Kinin-hormones" would be not to have special glands of secretion, being released from inactive precursors (bradykininogen, angiotensinogen) or from diffuse stores in the intestinal tract or the central nervous system (substance-P). On the other hand, the suffix "kinin" preceded by the name of the original source might be useful to denote the polypeptides of uncertain nature spotted in the venoms of the wasp (vespakinin) or of the scorpion (scorpiokinin) or of the hornet (hornetkinin). Also the principle excreted in the urine and displaying bradykinin-like activity, might be properly designed as "urokinin". Such varied nomenclature might, of course, one day be simplified as our knowledge of the chemical consistitution of the different mentioned principles progresses.

582 Structure and Actions of Pure Bradykinin. D. F. Elliott and G. P. Lewis (United Kingdom).

Bradykinin is formed when trypsin or snake venom acts upon the pseudoglobulin fraction of blood proteins. The peptide formed by the action of trypsin is the same as that formed by the action of snake venom and is a nonapeptide—Arg.Pro.Pro. Gly.Phe.Ser.Pro.Phe.Arg. Bradykinin has been synthesized and the chemical and biological properties of the synthetic material are identical with those of the natural substance.

Pure bradykinin has five pharmacological actions —stimulation of smooth muscle structures, vasodilatation, increased capillary permeability, accumulation and migration of leucocytes and pain production. The biological properties of other peptides will be compared with those of bradykinin and the role played by bradykinin in physiological and pathological reactions will be discussed in relation to its pharmacological actions.

583 Synthesis of Bradykinin and of Structurally Related Peptides. R. A. Boissonnas (Switzerland).

Immediately after the disclosure by Elliott *et al.*[1] of the octapeptidic sequence (H-Arg-Pro-Pro-Gly-Phe-Ser-Phe-Arg-OH) they initially proposed for bradykinin, the author's research group performed by several routes the synthesis of this octapeptide which was not only shown to be devoid of biological activity but also to behave differently from bradykinin toward chymotrypsin. Starting from the assumption that the proposed sequence was probably incorrect and that an error was more likely to be present in the middle than at the ends of it, the author's group undertook the systematic synthesis of the more probable alternative structures. One of the octapeptides so synthesized (H-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH) was found to possess a small bradykinin-like activity. This prompted the author's group to prepare the nonapeptide (H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH), which was found to possess all the biological and chemical properties of natural bradykinin.

1. Elliott, Lewis and Horton (1960), *Biochem. J.*, **76**, 16P.

584 Actions of Bradykinin-like Synthetic Polypeptides. H. Konzett and E. Stürmer (Austria).

585 Bradykinin and Vaso-Dilating Polypeptides. Antagonists of Bradykinin. H. O. J. Collier (United Kingdom).

Antagonism to bradykinin is being studied because (1) specific antagonists might help to elucidate the physiological or pathological functions of this and related kinins, as antihistamines have helped to clarify the role of histamine; (2) the possession of antibradykinin activity by known drugs might help to explain their mode of action; and (3) antibradykinins might have therapeutic value. Antagonism to bradykinins is only one aspects of the relation of drugs to the release, activity and destruction of kinins.

In the guinea-pig, antipyretic analgesic drugs potently antagonize bradykinin in its bronchoconstrictor action.[1, 2] This antagonism is reversible by higher doses of bradykinin and is specific, the drugs being effective at doses that do not affect bronchoconstriction due to histamine, acetylcholine or 5-hydroxytryptamine. Likewise, antagonists of histamine, acetylcholine and 5-hydroxytryptamine do not suppress bradykinin bronchoconstriction. Collier and Shorley[2] showed that the order of potency of analgesic antipyretic drugs as antibradykinins corresponds fairly closely with that found by Winder, Wax, Burr, Been and Rosiere[4] for the suppression of skin etythema caused by ultra-violet radiation in the guinea-pig.

Although Lecomte[5] has reported that high doses of phenylbutazone antagonize the hypotensive action of bradykinin in the rabbit, we have found it difficult to show specific antagonism to actions of bradykinin other than bronchoconstriction, such as

hypotension in rat and guinea-pig, skin wealing in guinea-pig and constraction or relaxation of isolated pieces of intestine. These results suggest that at least two different types of receptor for bradykinin occur in tissues. So far, antagonism can be readily shown at only one of these types—receptors in the smooth muscle of the respiratory passages.

1. Collier, H. O. J., Holgate, J. A., Schachter, M. and Shorley, P. G. (1959), *J. Physiol., Lond.*, **149,** 54.
2. Collier, H. O. J., Holgate, J. A., Schachter, M. and Shorley, P. G. (1960), *Brit. J. Pharmacol.*, **15,** 290.
3. Collier, H. O. J. and Shorley, P. G. (1960), *Brit. J. Pharmacol.*, **15,** 601.
4. Winder, C. V., Wax, J., Burr, V., Been, M. and Rosiere, C. E. (1958), *Arch. int. Pharmacodyn.*, **116,** 261.
5. Lecomte, J. (1959), *Acta allergologica*, **14,** 408.

586 Bradykinin and Other Capillary Active Factors. M. Schachter (United Kingdom).

Bradykinin and serum kallikrein are among the most active substances in increasing capillary permeability, and their effects are extremely similar in this respect. When injected intradermally into guinea-pigs with circulating pontamine blue dye, both produce lesions of a more intense blue colour than those produced by histamine, and blueing appears within 2–3 min of intradermal injection, whereas the delay with histamine is 4–6 min. They are both more effective wealing agents than histamine in the lower concentrations, and their dose-response curves differ from that of histamine in that they show a pronounced flattening with higher concentrations. Their effects are unaffected by doses of mepyramine which suppress the response to histamine.

Our results suggest that this action of kallikrein is due to the release of kallidin, with which bradykinin appears to be identical (Holdstock, Mathias and Schachter, 1957[1]). Kallidin also increases capillary permeability and its effect is like that of bradykinin.

Plasmin has little or no action on capillary permeability, and it releases little or no kinin from plasma. Our results suggest that kallikrein, and not plasmin, is the active agent in plasma which releases kallidin.

1. Holdstock, D. J., Mathias, A. P. and Schachter, M. (1957). *Brit. J. Pharmacol.*, **12,** 149.

587 Some Effects of Bradykinin on the Central Nervous System. R. Čapek (Czechoslovakia).

No attention has been paid to the action of bradykinin in the central nervous system. In the present experiments behavioural changes after the intraventricular injection of bradykinin in mice and cats and interaction of bradykinin, leptazol and strychnine were investigated.

The intraventricular injection of bradykinin in cats causes typical changes of behaviour. The animals have motor disturbances, muscular twitches, they lie down in bizzare positions and, finally, mainly tonic, exceptionally clonic convulsions occur. Vegetative functions are markedly altered (hyperventilation, salivation, micturition, defecation, mydriasis). Slightest touching causes cintinuous miaowing. After 60–90 min, the animals become normal again. After intracerebral injection in mice there is motor excitability, pilomotor reaction, tachypnoea and often convulsions.

As may be expected from the previous results, bradykinin after intravenous injection increases the convulsive action of leptazol and strychnine. These results are further discussed.

588 Pharmacology of the Lung. Introductory Remarks. G. Liljestrand (Sweden).

589 Effects of Drugs on Pulmonary Vessel Tone. E. Berglund (Sweden).

590 Influence on Pulmonary Circulation of Acetylcholine. B. Söderholm (Sweden).

591 Influence on Pulmonary Circulation of Serotomin. G. Bojs (Sweden).

592 Influence on Pulmonary Circulation of Histamin. H. Westling (Sweden).

593 Influence of Drugs on Bronchial Circulation. D. Aviado (U.S.A.).

594 Effects of Dust and Drug Inhalation on Airway Resistance and Lung Capacity. C. Heymans and L. Dautrebande (Belgium).

595 Influence of Drugs on Bronchiolar Smooth Muscles. H. Konzett (Austria).

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